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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. NO. 81

ON SENSORY ACTIVATION BY ALKALIES

W. J. CROZIER

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I. Weak acids and weak alkalies have in general a more powerful physiological action than would be predicted from their ionization. The explanation of this condition depends in part upon the fact that weak acids and weak alkalies are known to penetrate cells with relative ease (9). It is possible to utilize the observed relative speeds of cell penetration by different acids and alkalies in attempting to account for the manner in which these substances act upon receptor organs of the chemical sense. If an increase in the permeability of the cell surface is a frequent or an invariable concomitant of the process of excitation, it is important to discover in particular instances the rôle played by this change in permeability, as well as the manner in which the change is produced. It should be possible to obtain some light upon this matter through the study of receptor organs which may be normally activated by direct chemical means.

The present experiments were made in order to compare the stimulating powers of NaOH and NH_4OH, the former representing the strong alkalies which penetrate cells with difficulty, the latter a weak alkali entering cells with ease (6), (5). The animals used were earthworms, Allolobophora sp., obtained from a "fertilizer" pit containing a large amount of earth and vegetable material together with a small proportion of manure. The method of stimulation has been described previously (3); the worms were placed, one at a time, upon a low

ridge separating two shallow tanks, one of these containing an activating solution, the other holding boiled rain water. At the moment of adjustment, the worm was situated with one-half in the stimulating solution, the other end being in water. The stimulated part was, as a result, caused to be pulled into the water. The time occupied by this movement, that is, from the instant of adjustment in the activatting solution until the part concerned had been retracted from this solution, was measured with a stopwatch.

The interval so timed is regarded as an indication of the intensity of the activation of the worm under the particular conditions. When precautions are taken to standardize the procedure, using worms of uniform size and previous history, it is possible to obtain in these experiments "retraction-time" figures which are, to a fairly high degree, reproducible in successive experiments.

In these tests the *posterior* half of the worms was stimulated, since it was desired to eliminate consideration of the special sensitivity of the prostomium, and, in addition, to apply to the gross "retraction-time" figures as measured a correction increasing their significance. This "correction" consisted in taking account of the fact that stimulation of the posterior end of the worm (when not too intense) merely increases its normal tendency to move in an anterior direction; while there is an appreciable minimum time required for the fastest possible retraction of the worms; this correction seems legitimate because only one principal type of motor response is being considered (which is not the case with anterior stimulation according to this method). For most purposes it proved sufficient to subtract from each average "retraction-time" the average shortest interval required for retraction, since this factor would be appreciable only under conditions of rapid movement following strong stimulation of the worm. The percentage increase in rapidity of movement of the worm, induced by each stimulating solution, may also be calculated upon the basis of an ascertained average rate of progression when a special stimulant is absent; this procedure is less accurate than the former, and leads qualitatively to the same conclusions as the simpler method first outlined.

The "reduced retraction-time" figures are regarded as inversely proportional to the intensity of the activation experienced by the worms.

II. Average measurements of the "retraction-times" of the earthworm from solutions of NaOH and of NH_4OH are given in tables 1 and 2 (see also fig. 1). These figures are each the average of twenty

determinations. A single earthworm was used but once, thus avoiding "after effects." The averages were reduced, as previously described, by subtracting from each figure the minimum time required by these worms to effect the creeping movement of retraction. This amounted

TABLE 1

Retraction-time of earthworms from NaOH *solutions; concentration = N. × 10³; R.T. = retraction-time. 27°0.*

CONCENTRATION $N \times 10^3$	*R. T.*	$\frac{1000}{R.T.}$
	seconds	
62.5	5.00	200.0
50.0	5.40	185.0
37.5	7.25	138.0
25.0	11.6	86.2
12.5	18.9	52.9
6.2	44.3	22.6
5.0	50.0	20.0

TABLE 2

Retraction-time of earthworms from NH_4OH *solutions; concentration = N. × 10³; R.T. = retraction-time. 27°0.*

CONCENTRATION $N \times 10^3$	*R. T.*	$\frac{1000}{R.T.}$
	seconds	
125.0	4.11	244.0
119.0	4.80	208 0
95.3	6.62	151.0
81.7	8.50	118.0
61.2	11.2	89.2
59.5	11.3	88.5
50.0	13.8	72.5
40.9	19.8	50.5
31.2	17.1	58.5
30.6	15.6	64.5
25.0	26.7	37.5
20.4	31.9	31.4
15.3	40.6	24.6

to 1.3 seconds, and was obtained from tests made with worms stimulated several times in quick succession; the correction figure cannot be obtained from experiments with concentrated solutions because of their toxic effect.

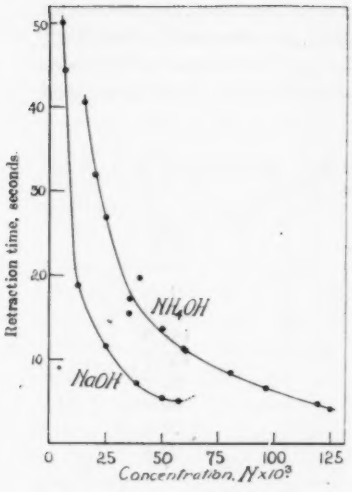

Fig. 1. Retraction-time of earthworms from alkaline solutions. See tables 1, 2.

The solutions employed were prepared by finding a maximum concentration of each alkali which would permit normal escape of the worms without producing obvious toxic consequences. Dilutions were then made from such a solution, analyzed by titration, and tried in succession.

Within the limit of error imposed by the nature of these experiments, the results agree satisfactorily with the requirements of the principle of mass action. For each alkali, over a considerable range of concentrations, the product of the concentration of alkali by the (corrected) "retraction-time" is sensibly constant. The individual observations deviate from the rule to the extent shown graphically in figure 2, where the logarithm of the concentration is plotted against logarithm $\frac{1000}{\text{R. T.}}$. The average "mass action constants" were used in drawing the straight lines shown in figure 2; the slope of these lines is 45°.

If the degree to which the earthworm is stimulated by alkali depends upon the amount or concentration of a substance, S, formed in its receptors through the action of alkali upon some receptor constituent, the rate of formation of S should be proportional to the concentration of alkali, according to the law of reactions of the first order. In the present instance, the intensity of stimulation is

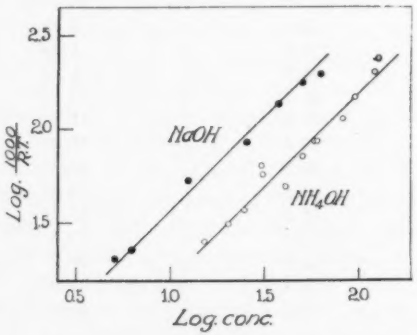

Fig. 2. The stimulating efficiency of alkaline solutions (measured by the reciprocal of the retraction-time of the earthworm), plotted against concentration of alkali.

assumed to be directly measurable by the reciprocal of the (corrected) "retraction-time," hence the product (retraction-time) × (concentration of alkali) should be constant for each alkali, as is found to be the case.

It should especially be noted that the "time" here considered does *not* have the significance of t in the reaction velocity equation. This "time" is, it is true, roughly equivalent to the period during which the worm has remained in the alkaline solution; and from this standpoint a parallel might be suggested between the outcome of these experiments and such a condition as that described by Lillie (8) with regard to the activation of Asterias eggs by butyric acid. In each of these instances a definite physical result appears: the starfish eggs form a normal fertilization membrane, the earthworms move out of the stimulating solution; and in both cases the time required to effect this physical result is inversely proportional to the concentration of the activating agent (within physiological limits). But this analogy is readily seen to be inadequate. When the worm is placed in alkali it begins, after an interval which varies with the concentration of alkali, to creep forward, and the "retraction-time" as measured with the stopwatch includes this period as well as a following one during which (at a rate depending upon the activity of the worm) a gradually, decreasing length of the animal is being exposed to the action of the alkali; the sensitivity of the skin of the earthworm is different at different axial levels. For these reasons the "retraction-time" may not be regarded as a measure of the time of action of the alkali. There is some reason to believe that the actual period of stimulation may be very brief indeed, and amount only to a fraction of the total "retraction-time" (except for the highest concentrations used).

On the other hand, the constancy of the relation illustrated in figure 2, which is significantly displayed also in the effects of acid solutions (cf. the following paper), fully justifies the contention that the intensity of activation of the earthworm is directly proportional to the acting concentration of alkali.

From this fact alone it cannot be concluded that a "monomolecular" reaction is at the basis of stimulation in this case. The same equation applies in other heterogeneous reactions,[1] such as the solution of a

[1] The stimulation of the skin of an earthworm by immersing the animal, or a part of it, in a solution brings into play several sources of "heterogeneity." The earthworm is covered with a resistant cuticle pierced by nephropores, gland cell openings, and numerous apertures through which access is had to the spe-

metal plate by acid; in that case hydrodiffusion of acid (governed by the concentration of acid in the body of the solution) is the slowest, or limiting, process the speed of which is measured in estimating the course of the reaction (13). Such a process has, however, a low temperature coefficient, that of hydrodiffusion. Some figures given by Shohl (21) point to a temperature coefficient of $Q_{10} = 2 +$ for the stimulation of the anterior end of the earthworm by alkali (NaOH); this finding I can confirm, for the posterior end, from experiments with NaOH and NH_4OH in which the observations were corrected for the effect of temperature upon the rate of locomotion of the worms, which were at the same temperature as the activating solutions (20).

It may therefore be suggested that sensory activation in this case depends upon a reaction between the alkali and some constituent of the receptor cells. It is to be supposed that this reaction is "reversible," and that it conforms to the law of reactions of the first order.

"Monomolecular" effects of this type are encountered in the measurement of toxicity (19), (16), and in the action of salts on protoplasmic permeability (14); apparently the action of NaOH in producing an increase in the permeability of protoplasm also follows this law, according to Osterhout's measurements (15). A similar interpretation has been put upon the course of erythrocyte haemolysis by bases (Arrhenius, cited by Lillie, (8)).

The fact that the response which is here regarded as a measure of the intensity of stimulation does not follow the Weber-Fechner rule,

cialized distal ends of sensory cells. These sensory cells are presumed to be concerned in chemoreception, since when *small* areas of the worm's surface are stimulated the reaction time of the worm varies inversely with the number of sense organs in that area (1). Whether or not all the sense organs in the posterior region of the worm are activated by immersion in alkali, cannot be decided, but it seems probable that a sufficient number of them is always stimulated to overcome the objection that the number of sense organs activated determines the speed of locomotion of the worm (as might be the case if the "all or none" principle were applied). This objection is likewise combatted by experiments in which varying lengths of the worms were immersed in alkali. These tests gave no indication that the degree of stimulation effected is proportional to the total area of the worm acted upon. The "specific excitability" of the worms varies for different regions of the animal's surface, as previously stated, but no evidence has been obtainable, from a great number of tests with various substances, that there exist any specializations into different kinds of chemoreceptors (such as the salt-, acid- or bitter-sensitive organs on the human tongue), but it is possible that in some cases free nerve terminals of a 'common chemical' sense are concerned in stimulation.

need cause no inconvenience in this connection, since we know of other instances where the motor result is directly proportional to the physical intensity of the activating agent—such as those photic reactions which obey the Roscoe-Bunsen law, (11), (18). Weber's rule appears to hold particularly when it is a question of the balance or discrimination between two stimulating intensities acting either simultaneously or in quick succession, upon the same receptive area; for example, in certain kinds of salt antagonism (10), (17), or, in the case of the eye, when the retina has become "adapted" to one light intensity before being acted upon by another.

III. It remains to compare the stimulating powers of NaOH and NH_4OH; the latter is much more effective than if the C_{OH} external to the worm were the determiner of activation. This difficulty is similar to that arising in connection with other physiological actions of ammonia, concerning which some complicated explanations have been advanced (12). It is well known that ammonia easily penetrates to the interior of cells. From Harvey's measurments (6) it appears that, on the average, at equal concentrations, NH_4OH penetrates cells ninety to one hundred times more rapidly than NaOH; while in a purely chemical action, such as the saponification of esters, the activity of NaOH may be as much as two hundred times as great as that of NH_4OH (measured by the amount of ester saponified).

If the activation of a sense organ by alkali be proportional to the extent of chemical change induced by the stimulating agent, then we might expect the amount of receptor material transformed to be proportional to the chemical activity of the base and inversely proportional to the difficulty experienced by the base in penetrating the surface of the cell. Assuming the receptor cells not to differ greatly from the generality of cells so far as their penetrability is concerned, we might expect in the present case to find NaOH two to three times as effective as NH_4OH. The extrapolated stimulating powers of unit concentrations of NaOH and of NH_4OH, that is, the "mass action constants" in figure 2, are in the ratio 2.4:1. This is consistent with the assumptious above made, since NH_4OH diffuses very rapidly into the surface layer of the cell, while the action of NaOH must be restricted to the very outer surface of the receptor (6), (2)—hence slow diffusion processes do not hinder the clearness of the result.[2] The

[2] Harvey's data (7) on the penetration of cells by strong hydroxides suggest that the speeds of penetration (neutral red method) are nearly proportional to the square of the concentration. The actual time-intervals involved are long, and probably intracellular diffusion complicates the phenomenon.

whole process of stimulation must take place at, and in, the surface of the cell, since the time of action is so brief.

SUMMARY

The chemically sensitive surface of the earthworm is acted upon by NaOH and NH_4OH in such a way that for each alkali the degree of activation, measured by its effect upon the resulting movements of the worm, is directly proportional to the concentration of alkali. Reasons are given for regarding this fact as evidence that a chemical reaction with some portion of the surface of the receptor elements is the essential feature of stimulation. From this point of view an increase in the permeability of the receptor cell surface, if it occurs,[3] is to be regarded as a consequence of activation, and not the essential determiner of stimulation.

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[3] According to Gray (4, p. 496), ammonia may penetrate sea urchin eggs, and give visible evidence of interaction with the cell pigment, although the conductivity of the eggs remains unchanged.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. NO. 82

SENSORY ACTIVATION BY ACIDS. I

W. J. CROZIER

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I. This paper deals with experiments undertaken to determine in a quantitative manner the relative effects of different acid solutions upon organs of chemical sensitivity. In order to secure some idea of the method of sensory activation by acids, comparisons are made with observations regarding the penetration of cells by acids. The animal used in the experiments upon the activating effects of acids was a common earthworm, Allolobophora sp., obtained in heaps of manure and decaying vegetable material. The method of experimentation has been described previously (6). The stimulation figures obtained in the present tests have to do with the activation of the posterior end of this worm and are not directly comparable with such data as those previously given (4) for somewhat similar tests made upon the anterior end of the worm. Detailed work has been limited to posterior stimulation, because in this way a uniform type of response forms the basis of measurement, and the special sensitivity (to osmotic differences, for example) of the prostomium and of the semi-protrusible buccal epithelium is at the same time eliminated from consideration.[1]

The assumption is made, in interpreting the measurements of time occupied by the retraction of the posterior half of the worm stimulated by immersion in acid, that the reciprocal of the average "retraction-time," which varies systematically with the concentration of acid, is directly proportional to the degree of activation of the worm. By "retraction-time" is meant the average observed time (in seconds) required for retraction from the stimulating solution, corrected by the subtraction of a figure representing the "impedence" of the worm,—

[1] The earthworm and the foot of the spinal frog are perhaps the most favorable for quantitative experiments of this nature, but it is necessary to point out that for the interpretation of results from these two sources somewhat different considerations are required.

the mechanical resistance to, or disadvantage of, its method of progression. The "correction" is obtained from experiments designed to show the minimum time required for the fastest possible retraction of worms of the constant size used in the stimulation experiments.[2] The correction was found = 1.2 to 1.3 seconds, at the temperature of these experiments (27°).

In using this method it is not possible to study the action of solutions which lead to retraction-times greater than would, on the average, be due to the normal locomotor speed of the worms under the given conditions, about 65 seconds,—although it is, of course, possible that under some conditions, such solutions should stimulate. The minimum working retraction-time is therefore about 2 seconds, the maximum about 60 seconds. But between these limits certain characteristic features of activation by the different acids used are sufficiently made clear.

Two series of acids were considered. Certain other series will subsequently be reported upon. In the first set of experiments the chloroacetic acids were compared with acetic and with hydrochloric, and in the second, the activities of monobasic fatty acids were compared.

TABLES

NOTE: In each of the following tables, excepting Table 5, *Conc.* signifies concentration × 10^3N; *R.T.* means the corrected average retraction time, in seconds.

TABLE 1

Monochloroacetic

CONCENTRATION	*R. T.*
59.8	0.7
29.9	1.2
25.0	1.4
15.0	2.2
12.0	4.5
10.0	6.9
6.0	21.7

TABLE 2

Dichloroacetic

CONCENTRATION	*R. T.*
29.0	1.0
14.5	1.3
8.7	5.5
7.3	4.8
5.8	5.7
4.4	25.4
2.9	45.0

TABLE 3

Trichloroacetic

CONCENTRATION	*R. T.*
13.0	1.6
10.4	1.2
7.8	3.7
6.5	4.0
5.2	4.1
3.9	28.0
2.6	40.0

[2] This minimum time is not always identical with the quickest retraction time observed with increasing concentrations of acid; the retraction time *vs.* concentration curves frequently pass through a minimum point, the lengthening retraction periods at higher concentrations representing the incidence of new types of response, such as writhing movements; very toxic solutions also retard the speed of movement, and frequently result in autotomy, (10).

TABLE 4

HCl

CONCENTRATION	*R. T.*	$C \times R.\ T.$	$C^2 \times R.\ T.$
26.6	3.8	101	
22.1	5.2	115	
17.0	6.0	111	
16.6	7.1	118	
14.8	7.4	110	
11.0	9.3	102	
8.3	12.5	104	
8.0	13.4	108	
6.6	17.6	118	(768)
5.5	25.7	(141)	780
4.4	40.8	(179)	792
3.7	57.0	(211)	781
Mean		110±5	(784)

II. Tables 1 to 4 and 7 contain a summary of the results obtained with the chloroacetic acids, hydrochloric, and acetic acid. These data (excepting that for acetic) are plotted in figure 1. The procedure consisted in finding by trial the highest concentration of each acid which would produce normal retraction without entailing immediate toxic consequences. Dilutions were then made from this concentration, and analyzed by titration. The retraction-times listed in the tables are each the average of twenty-five independent observations on twenty-five different worms.

There is a certain unevenness in the data for di- and trichloroacetic, which is greater than that found with the other acids, and has repeatedly appeared in other series of tests not here recorded. This may be due to the very rapid increase of stimulating power with increasing concentration of acid (tending to magnify errors of the experiment), or may be directly due to the complex nature of the stimulation process with these substances. The curves are, however, sufficiently separate to show that the general order of stimulating efficiency is

$$\text{acetic} < \text{mono—} < \text{di—} < \text{trichloroacetic.}$$

This order is not preserved, but becomes significantly irregular, when solutions of equal hydrogen ion content are compared (fig. 2).

Over a considerable range the stimulating power of the chloroacetic acids increases more rapidly than the square of the concentration.

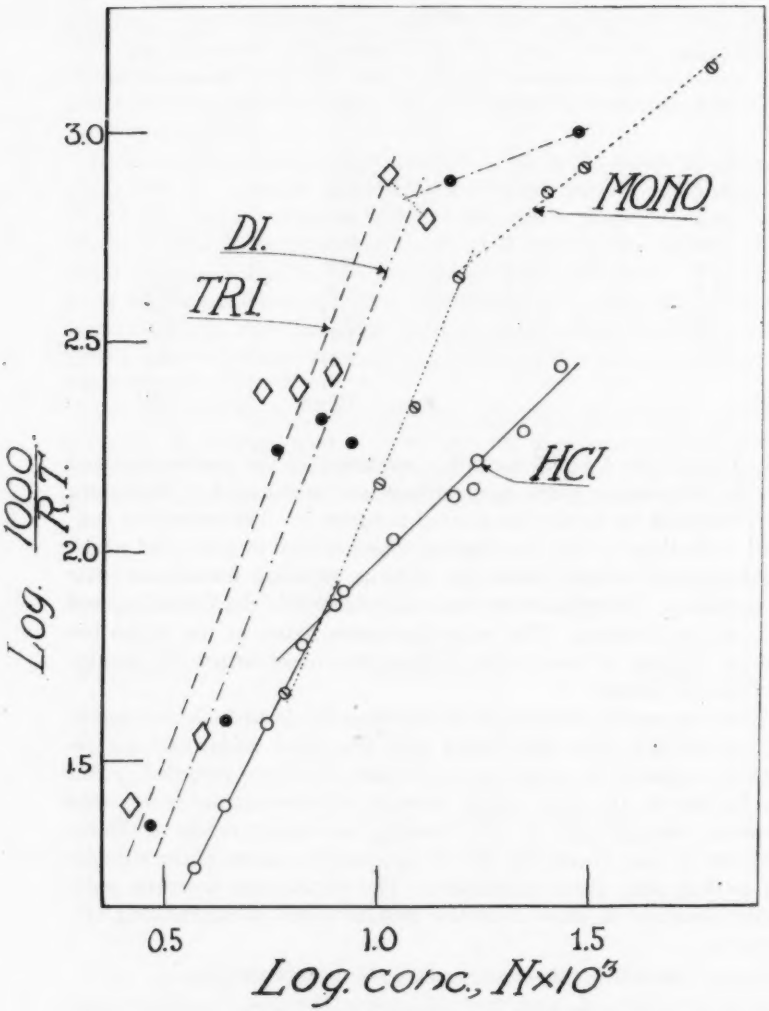

Fig. 1. Showing the relation between the reciprocal of the "retraction-time" of earthworms from solutions of the chloroacetic acids and HCl, and the concentration of acid.

In this respect they differ from acetic acid, and from the more concentrated solutions of HCl. The latter curves indicate, as in the case of bases (6), that chemical combination with some receptor constituent may be at the basis of activation. In the more dilute solutions of HCl the stimulating power is proportional to the square of the concentration.

It may be suggested that in the chloroacetic series several factors are involved in stimulation. Some light is thrown upon the nature of these factors by the following considerations.

a. From the quantitative study of cell penetration by acids it has been found that the speed of penetration of an acid is proportional to a fractional power of the acid concentration up to a certain concentration, beyond which the speed of penetration increases very rapidly. When plotted in the form

$$\log\left(\frac{1}{P.T}\right) = \frac{1}{n}\log C - \log K, —$$

where $P.T$ = the (corrected) penetration-time, C = the concentration of acid, and K is a constant for each curve (or part of the curve),—the resulting figures are, for each acid, characteristically composed of two intersecting straight lines.[3] The concentrations at which the acids begin to penetrate tissue with greatly augmented velocity are, in the case of HCl and the chloroacetic acids, significantly correlated with those at which they produce maxima in the viscosity curves of protein solutions. According to Pauli, the concentrations at which these acids produce maxima in the viscosity curves of albumen solutions are as given in the last column of table 5 (quoted from Ostwald (26)). Similar relations hold for other proteins. The second column of table 5 shows the concentrations at which an abrupt increase occurs in the speed with which these acids penetrate an indicator-containing tissue (Chromodoris). The first column of this table lists the maximal concentrations at which these acids could be used to stimulate earthworms; at concentrations higher than these, toxic effects resulted in less than 1 second, so that the worm did not escape from the acid solutions.

[3] The correction factors involved in this treatment are obtained directly (without assumptions) from the empirically determined penetration curves. A discussion of this matter will appear elsewhere. Some of the data are contained in previous papers (4), (5), but a good deal is as yet unprinted. The equation given above is derivable from the well known formula for adsorption at constant temperature; but it does not refer to the *adsorption* of acid, in the penetration experiments.

The parallelism in these series of figures is on all essential points complete, and demonstrates the implication of cell proteins in the process of activation by these acids.

b. The operation of at least two factors in sensory stimulation by these acids is seen in the way according to which the stimulating power is related to the hydrogen-ion concentration. The hydrogen-ion content of the acid solutions used, calculated from standard conductance data, is plotted, in figure 2, against the "retraction-time;" formic acid (observations in table 6) is included in this figure. It is found that for a given "retraction-time" (which is a reciprocal measure of the activating power) the hydrogen ion concentration required decreases in the following order:

$$HCl > di - > tri - > monochloroacetic > acetic$$

TABLE 5

Showing the maximal concentrations for stimulation of the earthworm (Stimulation), the concentration at which a rapid increase is observed in the speed of cell penetration (Penetration), and the lowest concentrations above which, according to Pauli, the ionizations of the corresponding protein salts are diminished (Effect on proteins). All concentrations = 10^3N.

ACID	STIMULATION	PENETRATION	EFFECT ON PROTEINS
Acetic	>100	100	>50
Monochloric	63	40	>50
Dichloric	31	23	20
Trichloric	13	8.3	10
Hydrochloric	28	24	16

The facts to which attention is directed in *a* and *b* can be understood upon the assumption that that characteristic of the chloroacetic acids which determines their capillary activity coöperates with the hydrogen-ion concentration in effecting stimulation, and that (in part, at least) this stimulation concerns proteins of the receptor surface. According to the ideas developed especially by Langmuir (12), the capillary activity and lipoid solubility of these acids are together and simultaneously determined by the nature and orientation of the component parts of the acid molecules at the surface of their (aqueous) solutions. That surface effects are primarily concerned, is indicated by the very brief time required for the process of excitation (cf. 4), as well as by the relative activities of the monobasic fatty acids (fig. 3) which are subsequently discussed.

It is conceivable that the effects here considered have reference to the complex construction of the cell surface, which is independently indicated by a great variety of considerations (1, p. 129); (19); cf. also (28). In this connection use may be made of the relative speeds of cell penetration by the acids. There is a uniformity in the results

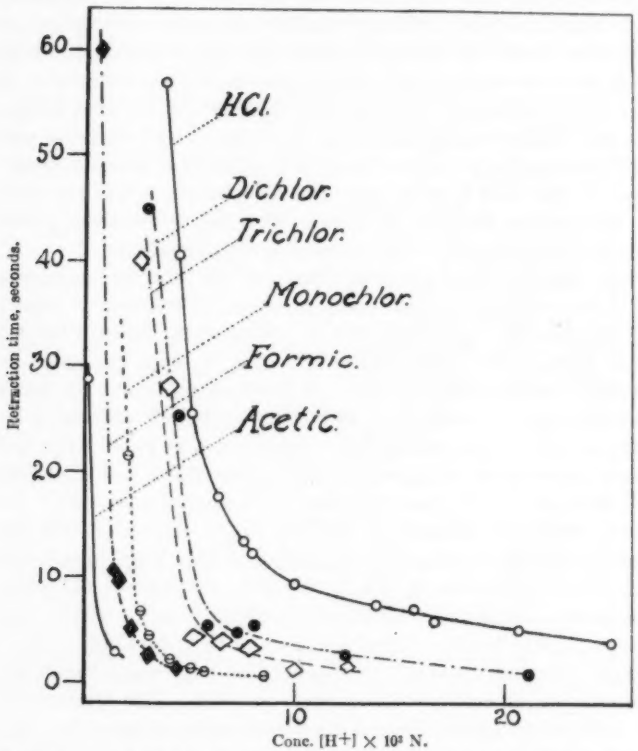

Fig. 2. Showing the relation between retraction-time and the (calculated) hydrogen-ion concentration of certain acid solutions.

which different observers have arrived at by this method (cf. (4), (9), (7)) which can only have reference to some general fact concerning protoplasmic organization. It is held that the penetration data afford a means of analyzing the surface composition of the cell, and that, according to the results of this method, both lipoids and proteins are

present at the cell surface. Hence it is possible that both the hydrogen ions *and* the remaining portions of the chloroacetic acid molecules are concerned in stimulation, and act upon both lipoids and proteins. The effect of these acids upon the surface tension of water, and their affinity for—solubility in—non-polar fatty substances depends, according to Langmuir and Harkins, upon the orientation of the molecules at the water surface in such a manner that the less active atomic groups are turned away from the aqueous phase. The surface activity of the chloroacetic acids increases in the same sequence as their ionization, so it is, then, not surprising to find that trichloroacetic acid, with highest ionization and highest surface activity, is (at equal hydrogen-ion concentrations) more efficient as a stimulating agent than dichloroacetic. In this way it can also be seen why the chloroacetic acids are more active in stimulation than is HCl, and why the stimulating power should increase very rapidly with increasing concentration.

This view requires that part, at least, of the activation process should include chemical action upon proteins. Preliminary experiments on the temperature coefficient of stimulation indicate for the chloroacetic acids a Q_{10} value (20°−30°) of 2 +, and the same for HCl; whereas in measuring the effect of temperature upon the speed of cell penetration by acids the coefficients obtained are about Q_{10} (20° − 30°) = 1.9 − 2.0, provided one considers that part of the acid curve where penetration is rapid, so that intracellular diffusion may be discounted,—at lower concentrations the coefficients are of the order of magnitude for diffusion or fluidity (Q_{10} = 1.1 − 1.7) (Cf. 22).

III. The comparative stimulating powers of the lower monobasic fatty acids, from formic to caprylic,[4] reveal interesting but not unexpected relations. Aside from formic acid, which is more active than valeric, these acids follow in general the order of their capillary activity and lipoid partition with water. Precisely these relations are seen also in the penetration of cells by these acids (4), (5). The figures contained in tables 6 to 12 are plotted logarithmically in figure 3.

It is seen that for each of these acids, excepting the lower concentrations of caproic and caprylic, the product (*Conc.*) × (*Retraction-time*) is essentially constant. With lower concentrations of caprylic and caproic acids the stimulating power is more nearly proportional to the square of the concentration. This may be the result of some "error" in the experimental method or it may be concerned with the

[4] The *normal* acids were used, except in the case of valeric, where the *iso*-acid was employed.

nature of the activation process itself. The phenomena in dilute caprylic and caproic solutions are therefore more comparable, it is believed, to that in dilute HCl solutions (cf. fig. 1) than to the curves of the chloroacetic acids. The squared-concentration effect appears in very dilute solutions, and does not appear in more concentrated solutions of other fatty acids of even much lower stimulating efficiency. One may therefore hold that the characteristic action of the weak acids is depicted by the constants derived from the $(C) \times (R.T)$ rela-

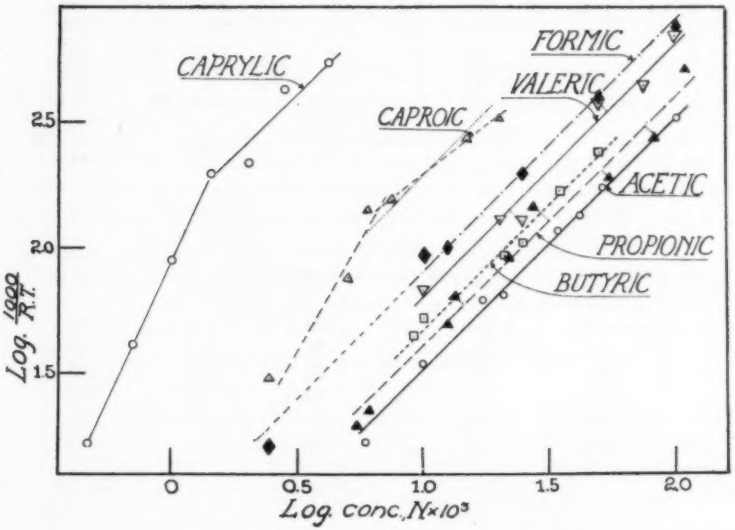

Fig. 3. Showing the relation between the reciprocal of the "reaction-time" of the earthworms from solutions of fatty acids and the concentration.

tions, or, in other words, by the portions of the curves (fig. 3) which slope at an angle of 45°.

These constants vary in a systematic manner with the effects of these acids upon the surface tension of water. There is, however, no ground for the supposition that surface tension changes, as such, are primarily implicated in stimulation. The behavior of HCl and of formic acid is significant at this point, as is also the fact (6) that NaOH is more active than NH_4OH. There is no indication that adsorption plays a part in the activation of the worms, as the stimulation curves

TABLE 6

Formic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
100	1.25	125
50	2.5	125
25	5.0	125
12.5	9.8	12.3
10.0	10.5	105
2.5	60	(150)
Mean		121±6

TABLE 7

Acetic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
103.5	3.0	310
52	5.8	302
42	7.4	311
34.6	8.5	294
21.	15.2	319
17.3	16.0	277
10.0	28.8	288
(6.0	60.0	336)
Mean		300±12

TABLE 8

Propionic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
110	1.9	209
82.5	3.6	297
55	5.2	276
27	6.8	184
22	10.8	240
13.5	15.4	208
12.5	19.8	248
6.1	44	268
5.5	59	(324)
Mean		241±31

TABLE 9

Butyric

CONCENTRATION	*R. T.*	*C.* × *R. T.*
50	4.1	205
35	5.8	203
25	9.4	235
21	10.6	229
10	18.8	188
9.2	22.1	203
Mean		210±13

TABLE 10

Valeric (iso-)

CONCENTRATION	*R. T.*	*C.* × *R. T.*
100	1.4	140
75	2.2	165
50	2.6	130
25	7.7	192
20	8.9	178
10	14.6	146
Mean		155±18

TABLE 11

Caproic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
20	3.0	60.0
15	3.6	54.0
7.5	6.4	48.0
6.0	7.0	42.0
5.0	(16.3)	
2.5	(32.5)	
Mean		50.8±6.0

TABLE 12

Caprylic

CONCENTRATION	*R. T.*	*C.* × *R. T.*
41	1.8	7.38
2.8	2.3	6.44
2.0	4.5	9.00
1.42	5.0	7.10
1.0	(11.1)	
0.7	(25)	
0.5	(60)	
Mean		7.48±0.6

(fig. 3) are not of the proper shape and indeed give evidence of a contrary significance. If adsorption (surface condensation) of acid upon the receptor organs were a deciding process in stimulation, we should expect to find that some function of the form $(C.^{1/n}) \times (R.T.)$ would be constant for different concentrations of any one acid; because in the commonly used equation

$$\frac{x}{m} = KC^{1/n}$$

m (the adsorbing surface) is by the method of experiment made constant, and because the degree of activation, measured by $\frac{1}{R.T.}$, would be proportional to x (the amount adsorbed),—or to a logarithmic function of x, in case Weber's rule were to apply. This is not found to be the case. It is doubtful if the ordinary adsorption equation can legitimately be applied to a matter involving such brief time-intervals, but in any event some similar expression, involving a fractional power of the concentration, would be expected; whereas in fact the amount of activation appears to be proportional to the concentration, or to the square of the concentration, or increasing even more rapidly than this. According to Lillie (14), (15), the activation of starfish eggs by butyric acid follows the same law as that found here in a case of sensory excitation.

Recent work on surface tension has demonstrated that the "capillary activity" of the fatty acids, as well as their effect upon the interfacial tension in such a system of immiscible phases as benzene-water, depends upon the orientation of the molecules and their orderly arrangement with respect to the surface of the water phase (Langmuir, (12) and Harkins, (8)). With the dilute solutions here studied the concentration of acid at the interface between water solution and the receptor cell, while higher than that within the body of the solution (or rapidly becoming so soon after the formation of this interface by the immersion of the worm), may nevertheless be considered proportional to the formal concentration of acid. The surface-activity of these acids, at corresponding concentrations, increases by a constant amount for each addition of CH_2 to the molecule, because "each CH_2 group, in these solutions, forms a part of the surface," and the potential energy of the surface is therefore correspondingly increased by a constant amount (cf. Langmuir's discussion of the data of Trauble and others, (12, p. 1885 et seq.). The stimulating power of these

acids should increase by a constant amount for each addition of CH_2 to the acid molecule (neglecting the effects of isomerism, which are relatively unimportant). Figure 4 shows that this expectation is realized. In curve *A* the abscissas represent the number of CH_2 groups in the molecule, from formic (0) to caprylic (7), the corresponding

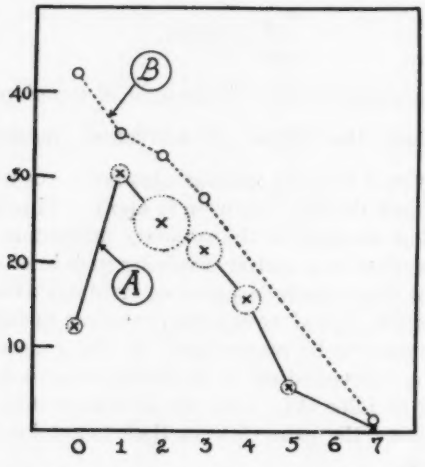

Fig. 4. *A*. The ordinates are the concentrations ($N \times 10^3$) required to effect a definite amount of stimulation (such that $\log \frac{1000}{R.T.} = 2.00$). The abscissas are the number of CH_2-groups in the molecules of the monobasic fatty acids, from formic 0 to caprylic 7. The dotted circles are drawn with a radius equal to the average deviation calculated from individual observations (cf. tables 6 to 12).

B. Against the same abscissas as in *A* there are plotted the times required to effect membrane formation in 60 per cent of the eggs of the sea urchin, according to experiments tabulated by Loeb (15), in acid solutions at 0.001 N concentration. The unit of ordinates is here = 0.1 minute (see text).

ordinates being the concentrations required to effect a definite amount of activation (such that $\log \frac{1000}{R.T.} = 2.00-$ see fig. 3). This curve shows that, aside from formic acid, the amount of each fatty acid which is required to bring about a constant degree of activation decreases in regular manner (within the limits of error of the observations) according to the number of CH_2 groups in the acid molecule.

The exceptional behavior of formic acid is due to its much stronger ionization.[5] It is probable that here the hydrogen ion is also concerned in activation. These acids may, in other words, combine with the surface of the cell, *a*, through the affinity of the carboxyl group for water or protein; and, *b*, through the affinity of their hydrocarbon chains for lipoids. When *b* is relatively much larger than *a*, owing to the orientation of surface molecules of acid, it alone appears to be the controlling factor in stimulation, as in the fatty acids other than formic. Hence there is no evidence among these acids that their ionization affects their stimulating ability, as in that event some disturbance of the straight line relation depicted in figure 5 would be found. Careful work may show that such deviation does in fact exist in some cases, and in human taste it is known that although the sourness of different acids depends directly on their tendency to ionize (when the penetration of the receptor is taken into account), the acids weaker than acetic nevertheless produce a more powerful sensory effect of another kind (and probably upon the same taste cells).

IV. Relations similar to those just described are found in comparing the penetration of cells by these acids. In referring these effects to interactions with cell lipoids, there are several objections to be considered.

a. It might be conceived that sensory cells specialized for chemoreception are merely more permeable, to acids, for example, than most cells appear to be. There is no good reason favoring this belief, and the reverse is equally likely to be the case. Low concentrations of acid do not behave in stimulation as high concentrations do in the penetration of cells in general. The chemoreceptors of the earthworm have long modified distal extremities, which project, cilia-like, through openings in the cuticula of the worm, and they must therefore be rather dense and rigid. (It has been suggested that these processes may contract upon stimulation, but it does not appear that surface tension forces thus brought into play are the determiners of activation.)

Chambers (2) states that a resistant surface film of a specialized kind is present only upon protozoans and germ-cells. The histological appearance of sensory cells suggests that they also have very specialized outer surfaces. It is, then, of interest to compare the effects of these

[5] Formic solutions have effects like those of HCl, and unlike those of the weaker acids, when their toxicity is considered. Formic acid is much more efficient in destroying the sensitivity of the earthworm's chemoreceptors than are the weaker acids.

acids upon other cells which do undoubtedly possess a highly specialized surface; such cells are found in the mature eggs of the sea urchin. The time required by solutions of the monobasic fatty acids, at constant concentration (0.001 N), to bring about activation (membrane formation) in 60 per cent of the mature eggs of the sea urchin is plotted in figure 4, curve *B*. The figures were obtained by graphic interpolation from data given by Loeb (17, p. 134). The ordinates for this curve are in units of 0.1 min. The ordinates for curves *A* and *B* are in different units, but are directly comparable because in the stimulation figures the concentration is inversely proportional to *R.T.*, which is itself inversely proportional (by assumption) to the intensity of stimulation; hence the "concentration" and "time" in the two sets of experiments have similar meaning. The behavior of formic acid is quite different in the two cases, but otherwise the effects are qualitatively identical. This suggests a difference in the superficial composition of the two kinds of cells which are compared.

b. Weak acids and weak alkalies have a more powerful action upon proteins than their H^+ or OH^- concentrations would theoretically warrant. In this respect there is a certain parallelism with the results of stimulation experiments, which might be regarded as evidence against the idea that lipoids are concerned in the stimulation effects. There is, however, independent evidence favoring the presence of lipoids at the surfaces of cells (cf. 19), and it seems unlikely that the relative activities of the several fatty acids could be accounted for on this basis.

On the other hand, Langmuir has shown (12, p. 1883) how the hydrogen ion may act to make thin oil (or lipoid?) films more mobile, and there are other physical effects of acids upon lipoids which might be considered significant. But the explanation of activation by the strong acids without reference to their action on proteins would ignore the curious parallels which have previously been pointed out. In addition, the stimulating power of HCl is greater than that of NaOH, which is also favorable to the idea of action upon proteins (cf. 29). Taking into account the very different behavior of the mineral and fatty acids in producing toxic results at high concentrations, and the differences in their behavior in penetrating cells, it seems most reasonable to refer otheir effects in stimulation to reactions with different components of the sensory cell-surface. Further experiments are being made to test this idea.

V. One view of the process of stimulation endeavors to reduce all

forms of activation to a common basis of increase in the permeability of the cell surface. A widely entertained theory of this nature, notably developed in the writings of R. S. Lillie, considers that the essential act in stimulation has to do with the depolarization of the cell surface, which is supposed to exhibit in its resting state a polarization resulting from differential permeability toward oppositely charged ions, and particularly from its impermeability for anions. This idea of the origin of bioelectric potentials is, however, confronted by peculiar difficulties of its own (cf. 18), and the general conception that stimulation (activation) is brought about by agents operating to increase permeability is further opposed by the fact that substances which (so far as we know) act primarily to decrease permeability are very efficient in stimulation. This matter can best be studied by means of measurements of the stimulating powers of various substances for organs of chemical sense, where the problem of excitation by external influences has some primary significance. It is furthermore of interest to note that in the case of the earthworm the sensory cells which are probably concerned in the effects here described, are neurones of the first order, and that the ganglion cell is in all probability stimulated directly by the dissolved substances.

Strong acids (HCl) produce a decrease in permeability toward ions, which is followed by an increase only after the elapse of a relatively considerable interval (at the concentrations we are dealing with); (cf. 24). The general and specific parallelisms between the behavior of cells composing tissues of the most varied origin on the one hand, and the sensory receptors of the earthworm on the other, in penetration and stimulation experiments respectively, makes it unnecessary to assume the existence of fundamentally exceptional structural conditions at the receptor surface. Hence it is improbable that acid has here an exceptional effect upon surface permeability for ions. If, however, the hydrogen ion should *hinder* stimulation by inducing an increase in surface polarization or by otherwise decreasing permeability, then we should expect to find that acids would be specifically less efficient as excitants in proportion to their ionization. In figure 2 it will be seen that apparently this is in part true; for example, at a given stimulating power the [H·] values decrease in the order *acetic* < *formic* < *HCl;* the explanation, of course, lies in the fact that in these acids the stimulating property does not concern merely the H·.

There is also the fact that HCl, which leads to an increase in permeability only after a pronounced decrease, is specifically more ener-

getic as an excitant than is NaOH, which produces only an increase in permeability (23). Figure 5 shows that at all corresponding concentrations of [H·] and [OH⁻] HCl is a more powerful excitant than is NaOH. (The data for NaOH are taken from the preceding paper; the two series of experiments were strictly comparable). This is equally true at dilutions where Na· and Cl⁻ are quite inoperative in pure salt solutions, and where indeed if they were significantly con-

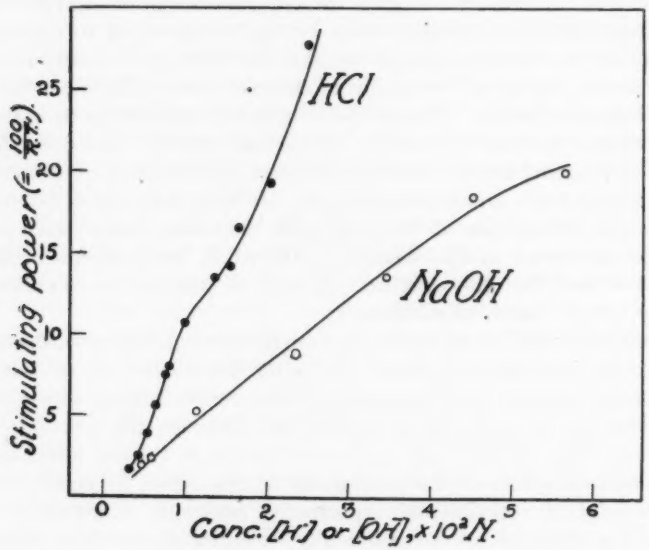

Fig. 5. The relative stimulating powers of solutions of HCl and of NaOH at corresponding concentrations of $H^{\cdot}$ and OH^{-}.

cerned a reverse order of stimulating capacities would be expected, since Na· is more powerful than Cl⁻ in activating these worms. HCl, moreover, is active at a lower threshold than is the alkali. These relations obtain also for the comparative toxic effects of H· and OH⁻, and are significantly seen also in taste excitation. The limiting dilutions at which HCl and NaOH are perceptible upon the tongue are respectively at [H·] = 0.0011 ± and [OH⁻] = 0.007 ± (11). According to Parker (27) the relative effectiveness of HCl and NaOH for

stimulation in lower vertebrates is of the same order.[6] It is significant that in Osterhout's experiments (23), (24) NaOH at 0.001 N concentration has practically no effect on permeability, although HCl 0.001 N has a pronounced effect in decreasing permeability. The limiting concentrations in the earthworm experiments were found to be: HCl $<$ 0.0037; NaOH = 0.005 $\pm$ (at the *anterior* end of the worm the absolute dilutions are greater, although the relative proportions are about the same). The changes in permeability measured by Osterhout are due to chemical reactions with surface constituents of the protoplasm.

Moreover, the concentration of HCl which in Osterhout's experiments with Laminaria induces a rapid increase in permeability (following the preliminary decrease) is between 0.015 and 0.02 N; that is, the extent of preliminary decrease in penetrability for ions reaches its maximum value in solutions about 0.015 N, and thereafter becomes smaller as the concentration of acid is made greater This is essentially the order of magnitude of the maximal concentration with which the earthworms in the present experiments may be stimulated and yet quickly recover. So there is reason to believe that, although the changes induced in specialized sensory cells by acids and alkalies are much more rapidly brought about than in ordinary tissue elements (and this is possibly the primary expression of their specialization), the essential nature of these changes is nevertheless identical in all cases (or in nearly all cases, excepting perhaps egg cells).

The results of experiments dealing with the activation of the earthworm seem, then, to be opposed to the idea that stimulating agents universally produce their effects by virtue of a permeability-increasing action. They are, however, favorable to the idea that in chemoreceptor activation there occurs some union, essentially chemical in nature, between the activating agent and some one or more constituents of the receptor surface (which may itself vary in composition according to the habitat of different worms of the same species). The amount of stimulation depends upon the character and extent of this combination. It is assumed that the "all or none" principle does not apply to these effects (which does not mean its lack of applicability to a single propagated impulse), since when large areas of the worms used are exposed to stimulation the intensity of activation is not pro-

[6] At higher concentrations the alkali is sometimes more stimulating; this is probably due to secondary influences, as different types of response may be concerned.

portional to the number of sense organs involved; in another direction it can be pointed out that human taste buds detect a wide range of differences in, for example, sourness.

These experiments are in agreement with a conception of stimulation which seems first to have been formulated by Loeb ((16) and earlier papers), which holds that ion-protein (or ion-soap) compounds control the ratios between free ions in the cell, and by variations in their composition or physical state determine in this way the propagation of impulses. Whether or not the specific form of this general theory elaborated by Macdonald (cf. 20) is applicable here, seems doubtful. In his theory a local colloidal condensation (or precipitate) at the point of excitation, decreasing the extent of local surfaces available for the adsorption of ions, results in a freeing of ions for diffusion. From these earthworm experiments it seems possible that either precipitation or the reverse may serve equally well for the initiation of excitation.

SUMMARY

When earthworms are stimulated by acids according to a method which gives quantitative results connecting the concentration of the excitant with the amount of stimulation induced, it is found that the acids stimulate as if by simple chemical combination with one or more constituents of the receptor surface. There are striking quantitative paralellisms between the powers of different acids to penetrate cells and the peculiarities of their relations in stimulation. This does not mean that they stimulate by mere diffusive penetration, but that similar combinations with cell materials are fundamental to both processes. Independently of assumptions made for purposes of quantitative treatment, the results of these experiments are inconsistent with the idea that activation is induced by surface depolarization of the cell, and with the associated, but unduly generalized, conception that stimulation involves in all cases an increase in cell permeability to ions. This conclusion is obviously not opposed to the idea that stimulated cells may in many cases become more permeable, but it does imply that an increase in surface permeability is not the determiner of activation. It does favor the idea that alterations in the condition of materials at the surface of the cell are instrumental in determining the diffusion of ions within the cell.

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X. DIFFERENCES IN THE BEHAVIOR OF SEGMENTS FROM DIFFERENT PARTS OF THE INTESTINE

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The writer has shown in a previous paper that, roughly speaking, the rate of rhythmic contraction in excised segments of small intestine varies inversely as the distance from the pylorus (1). Since then a better technic has been worked out. Five segments are now used in the same beaker of Locke's solution, and all are kept at a constant temperature of 38°. The rabbits are killed by a blow on the head; they are opened immediately and segments 15 cm. long are removed from the first portion of the duodenum, from the first portion of the jejunum, from the middle of the small intestine, from the lower ileum opposite the tip of the appendix and from the colon where it parallels the duodenum. These segments are kept in iced Locke's solution and shorter pieces, 2.5 cm. long, are cut as required. Long heart levers have been made with the arms more nearly equal, so that the magnification will be less and the five records will go on the drum. The measurements are 18.5 cm. from fulcrum to writing point and 12 cm. from fulcrum to thread. The lever is weighted just enough to keep the thread taut. The segments are held by small wire serrefines. The beaker contains 400 cc. of Locke's solution through which air bubbles.

Differences in tone. Differences in tone were observed in cutting the segments. The duodenal loops shortened a great deal after cutting and the ends rolled backwards, forming wide cuffs. The jejunal pieces contracted somewhat and showed narrower "cuffs." The middle pieces contracted still less and showed very little cuff formation. The ileum lengthened after excision and it showed poor tone. The colon showed even more tone than did the duodenum. It retained a very firm grip on the scybalae, and segments shortened to perhaps half their original lengths. These differences had to be taken into account so that the records would fit on the standard drum. The ileal segment had to be cut 1.5 to 2.0 cm. long and the duodenal and colonic ones

3.5 cm. long, so that they would be of equal length after immersion in the warm Locke's solution.

The greater tone of the duodenal and jejunal segments was shown also by the way in which they shortened after they began to beat rhythmically. The middle and ileal segments generally improved as regards amplitude of contraction, but the base line rarely rose. It generally remained very constant, probably because the muscle had relaxed until it was checked by the connective tissue along the mesenteric border. After attachment to the levers, the most pronounced rise in tone was almost always shown by the jejunal segment. The duodenal rise might have been even greater if the segment had not already contracted so much before immersion in the beaker. The colonic segments also shortened a good deal, generally after from ten to twenty minutes.

Comparative rhythmicity. The duodenal segment was generally the first to beat well. Sixty-eight records were examined as to this point and the different segments were credited with one, two, three or four points according as they began to beat first, second, etc. When two began to beat so nearly at the same time that no difference could be made out, they each received the same number. When the figures were averaged, the following data were obtained:

Duodenum	1.53
Jejunum	2.38
Middle	2.64
Ileum	1.70

The duodenum beat first forty-six times; the jejunum, thirteen; the middle, six; and the ileum, thirty-four. The great rhythmicity of the duodenal segments is the more striking when it is remembered that they seem to suffer most from trauma. They must be handled more carefully than the ileal segments. They apparently recover somewhat from the trauma of cutting during their stay in the cold Locke's solution because when attached to the levers immediately after removal from the animal, they were slower in starting up. The great vulnerability of this region probably accounts for the fact that in eight out of the sixty-eight experiments, the duodenal segment remained practically motionless. Even in sickly rabbits the other segments always showed some activity. Perhaps the high rhythmicity of the segments from the lower ileum is due partly to their comparative immunity from injury in cutting and handling.

The first few centimeters of the duodenum always beat very poorly with a small, variable amplitude. This agrees with the x-ray findings

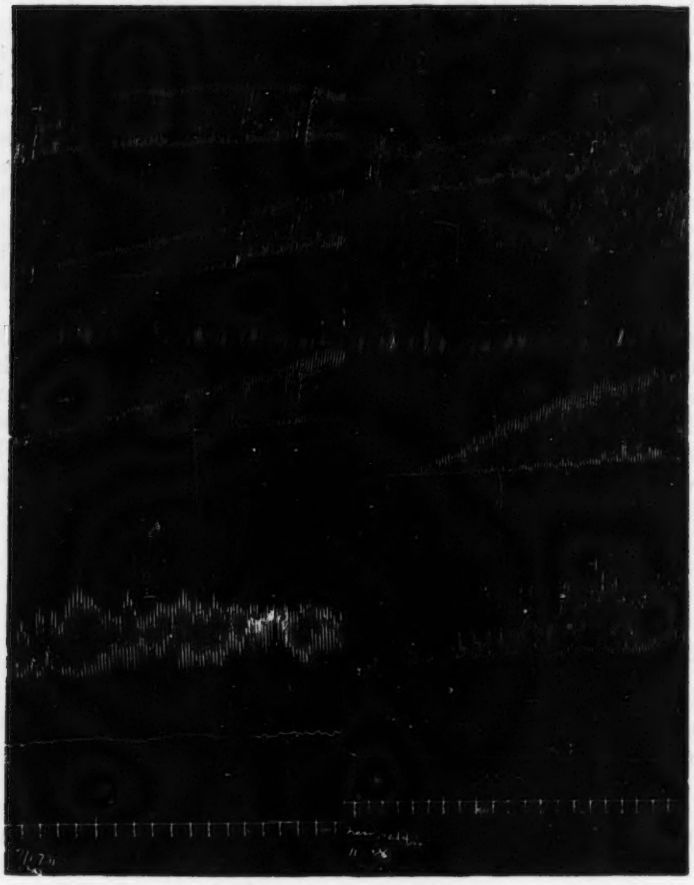

Fig. 1. Two typical beginnings. From above downward the tracings are from duodenum, jejunum, upper ileum, lower ileum and colon. The time record represents thirty seconds.

in man where the first portion of the duodenum ordinarily remains filled and shows little activity. The food naturally is delayed in this quiet region situated between two active ones.

The greater tendency to rhythmic contraction in the upper end of the tract is suggested strongly in figure 2. The segments were first poisoned with pilocarpine and then atropin was added. The first to escape was the duodenal segment and this was followed in order by the middle, ileum and colon. A similar graded escape from inhibition has

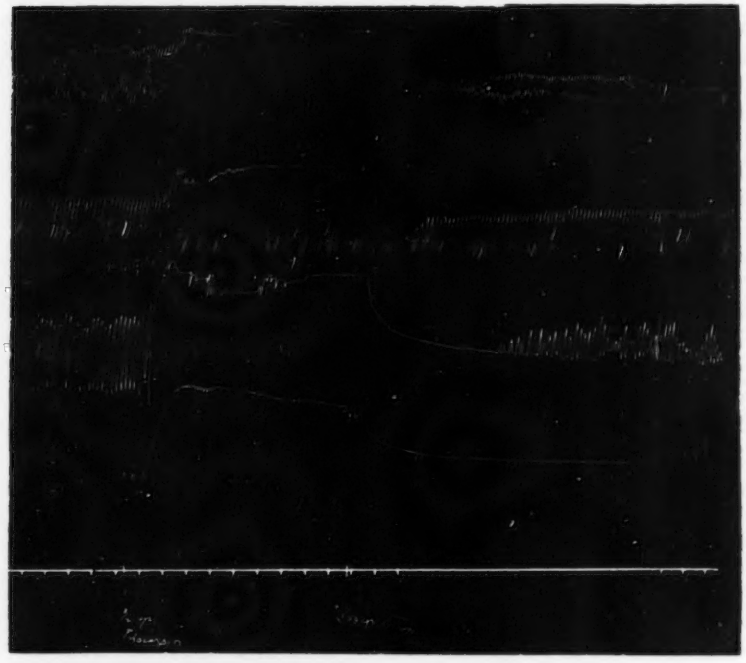

Fig. 2. To show the graded recovery from pilocarpin after adding atropin. From above downward, the records are from duodenum, upper ileum, lower ileum and colon. Time record represents thirty seconds.

been observed with some other drugs such as adrenalin and magnesium sulfate.

The colonic segments were so slow in beginning to beat that it was found best to cut them first and to leave them in the warm solution while the other segments were being prepared. After these were fastened to the levers, they were all lowered together into the solution.

Even with this head start, the colon often took an hour to get going well and it generally beat better on the second day after excision than on the first. The greater sluggishness of the colonic muscle was noticed also in pharmacological studies. Drugs which depressed the duodenum for thirty seconds often kept the colon paralyzed for five minutes or more. In some cases the drug caused a short drop and rise and then a permanent drop in the duodenum, but only the final drop in the colon. The contractions were very different, the rhythm irregular and the tone variable. The rate varied from 2 to 12 per minute. Unpublished experiments show also that the latent period of the colonic muscle is longer than that of the muscle in the small intestine. One cannot escape the impression that we have to deal in the small and large bowels with two very different types of muscle. The muscle in the colon acts more like the smooth muscle of a cold-blooded animal. The muscles in the small and large intestine, again, behave differently from the muscles in the body and antrum of the stomach.

Segments from sickly animals. The segments from sickly animals or animals heavily infected with parasites, generally beat poorly and irregularly. Sometimes they would begin beating well, or the first set would beat normally, but they generally became weak and erratic after a while. Sometimes segments from flabby looking intestines beat surprisingly well, with a very wide amplitude. The wide amplitude is probably a sign of poor tone (2). Ordinarily the duodenal and jejunal segments seemed to suffer most from the depression. Sometimes the duodenum would not beat at all even when the animal appeared to be pretty healthy. In some of the animals, short stretches of bowel were found to be flabby and filled with gas, while the rest of the gut appeared to be normal. It was interesting that when segments were excised from these peculiar regions they often failed to beat well, although the other segments behaved normally. Segments from rabbits whose abdomens were full of cysticerci generally beat well if the animal was well nourished and active. The colon did not seem to be much affected by the general depression and frequently it was the only segment that would contract normally. Segments of colon were markedly depressed in some animals with a tendency to diarrhoea. This agrees with x-ray studies in man which generally show the diarrhoeic colon unsegmented and flabby. The segments from the sickly animals often reacted poorly to drugs.

In one animal an inflamed Peyer's patch was noticed in the lower ileum. The inflamed segment, when excised and put into Locke's

solution, did not beat well and its rate was about normal. The segment just above, however, beat 21.5 to 25 times per minute. This

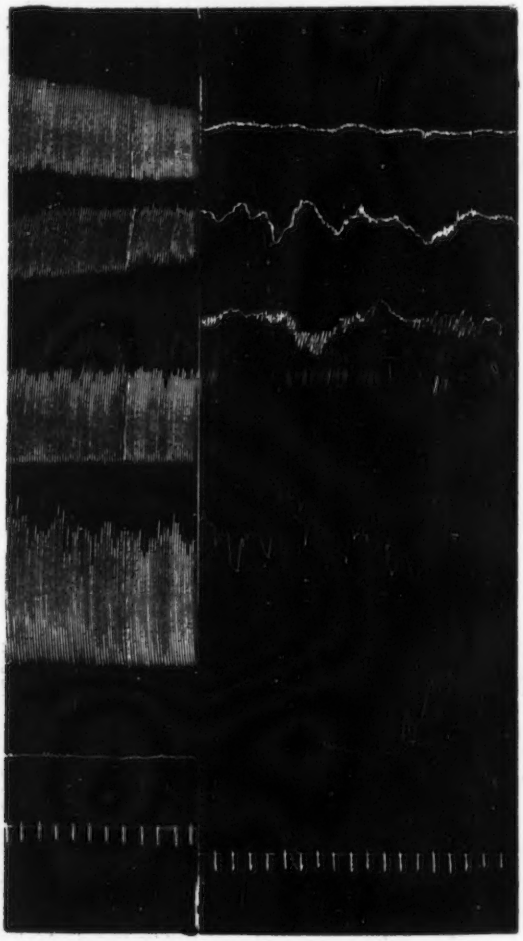

Fig. 3. Records from healthy and from diseased animals.

is not only twice the normal rate for the ileum but it is faster than normal for the duodenum. This shows what the writer has long sus-

pected, that inflammatory lesions can alter the gradients in the tract. These gradients may also be upset by the unevenness of the effects of disease toxins; that is, the duodenum and jejunum may be almost paralyzed while the ileum and colon remain active. Such a reversal was observed while studying the latent periods in different parts of the stomachs of distempered dogs (3). These observations may explain many of the digestive upsets in thin, run-down, nervous women; upsets for which no anatomical explanation can be found, but which straighten out promptly under over-feeding and rest.

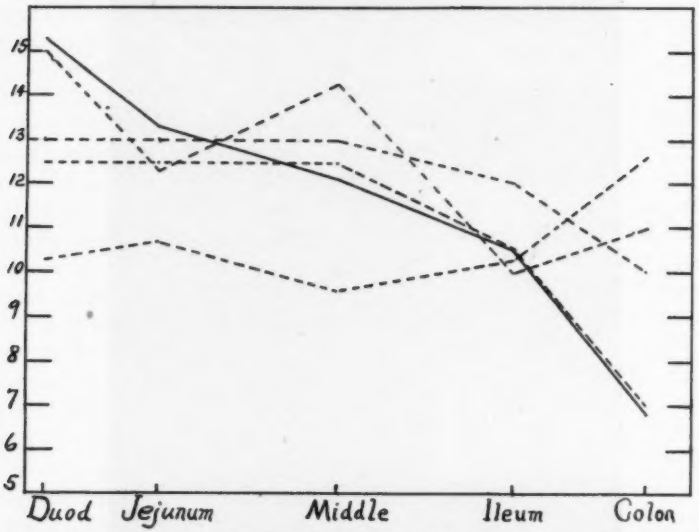

Fig. 4. Ordinates represent rates per minute; abscissae represent the segments at varying distances from the pylorus. The solid line represents the average for fifty-three animals. The broken lines represent data from sickly animals.

Gradient of rhythm. The rate of contraction has been counted in one hundred and seventy-six places on records from fifty-three rabbits. These animals were in good condition and the segments contracted well. The averages are as follows:

	per minute
Duodenum	15.3
Jejunum	13.3
Upper ileum	12.1
Lower ileum	10.5
Colon	6.8

These data have been plotted as a heavy line in figure 4, in which the ordinates represent rates per minute and the abscissae distances from the pylorus. Unfortunately, these abscissae can be only approximately correct.

It is interesting that in these fifty-three animals there was no instance in which the average of two or three readings on any one segment gave a figure higher than the average for the segment just above. The gradients were not so even, however, in the sickly animals. Samples of these are shown as broken lines in figure 4. These upsets in gradient have been found to be even more marked in the intact bowels of sick animals studied under salt solution (4).

Three animals were starved for three or four days (without muzzles). Their segments beat with a poorer amplitude than normal but no characteristic changes in rhythmicity or in gradient could be made out.

Behavior after twenty-four hours. It is a remarkable fact that after twenty-four hours in Locke's solution between 5° and 10°C., the segments beat faster and the gradient of rhythm was retained. This will be observed in the following three protocols:

	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
Duodenum	15.0	18.5	14.8	16.5	12.3	16.3
Jejunum	13.7	16.5	12.6	15.0	11.0	16.0
Upper ileum	11.9	14.0	11.0	12.0	8.9	14.3
Lower ileum	11.3	14.0	9.2	8.0	7.4	14.0
Colon	7.0	5.0			6.5	3.0

The strength of the contractions suffered and the segments often became fatigued quickly. They were also less sensitive to drugs although on two occasions they reacted typically with 1 part of adrenalin to 8,000,000 of the solution.

This evidence fits in with a great deal more (5) which it seems to me has proven that the rhythmicity is initiated in the muscle itself and not in the nerve-net. The differences in rate found normally in the different parts of the gut are due probably to differences in some phase of the metabolism in the muscle.

SUMMARY

Five segments excised from different parts of the rabbit's intestine have been studied under identical conditions in warm aerated Locke's solution.

The segments of duodenum and jejunum have greater tone and contract more after cutting than do the ileal segments. The colon also has a high tone.

The duodenal segment is generally the first to begin beating well. The tendency to rhythmic activity is graded from duodenum to ileum. The first few centimeters of duodenum, corresponding to the duodenal cap in man, does not beat well.

The colon is very slow in starting up and it differs greatly from the small intestine in its behavior.

The duodenum suffers more from trauma and from adverse conditions than do the other segments.

Segments from sickly animals beat poorly and become fatigued early. These changes are often more marked in some segments than in others so that the gradation of rhythm is changed.

The gradation of rate of contraction from duodenum to ileum is remarkably constant in normal animals.

In one case the gradation was upset by the presence of an inflamed area in the ileum. The bowel in that region contracted 21.5 to 25 times per minute, or twice as fast as normal.

After twenty-four hours, the segments beat at a faster rate and maintain the gradient. They continue to react normally to adrenalin and atropin.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

I. Relation of Oxygen Concentration and the Rate of Intracellular Oxidation in Paramecium caudatum

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So far as the writer is aware no attempt has ever been made to study with quantitative methods, the oxygen consumption of any unicellular animal. There are only two papers, Vernon (1) and Barratt (2) which attempt to give anything like measurements of CO_2 production by a protozoan cell.

The purpose of the present series of papers will be *a*, to describe methods which yield accurate and reproducible results on O_2 consumption and CO_2 production, and *b*, to show as far as may be, what the conditions are and how they affect the magnitude and rate of O_2 consumption and CO_2 production in these unicellular animals, using for this purpose to begin with, Paramecium caudatum. In this paper results are given to show what relation the concentration of oxygen has to the rate of intracellular oxidation.

METHOD

Preparation of Paramecium for experiment. The material for all the work was a pure line of Paramecium caudatum grown in large mass cultures in boiled hay infusion. The only other organisms present in the cultures were bacteria of various types commonly found in such infusions, which served as food for Paramecium; occasionally small amoebo-flagellates would occur which then served as part of the food supply for Paramecium. When the cultures were in their height of development the clear supernatant liquid containing the Paramecia was siphoned off carefully to avoid introducing any bacterial zoögloea. The organisms were then concentrated by use of the centrifuge at as slow a speed as possible. In this way injury to the animals was

avoided. The removal of the Paramecia to clear tap water, which whenever necessary had been sterilized by boiling, was accomplished gradually by repeatedly diluting the concentrated suspension with sterile or ordinary tap water at room temperature and then centrifuging. This transfer and washing of the Paramecia must ordinarily be gradual in order to allow time for the animals to adjust themselves to the new chemical and osmotic conditions. Transfer to pure tap water and washing was usually extended over a period of fifteen to twenty-five hours. In this way, with care, it is possible to wash Paramecia perfectly free from the native medium and to reduce the bacterial content of a Paramecium suspension to that of ordinary tap water or tap water which has been previously boiled. Hargitt and Fray (3) have shown by a series of careful tests that it is possible to sterilize a Paramecium by washing it five or six times in different portions of five to ten drops of sterile water. For the purposes of the experiments in this and following papers the small bacterial content of tap water and boiled water has no significance since if all the Paramecia remain alive, there will then be no pabulum in which bacteria in sufficient numbers to disturb the results will grow. Controls which rule out all effects from bacteria were always carried out whenever conditions demanded. These conditions will be referred to at the proper time. When Paramecia are washed in this way they are under starvation conditions. The food reserve of the protoplasm is gradually depleted as shown by the gradual decrease over a number of days in cell lipoids and increase in transparency of the protoplasm to light. But Paramecium will often live for as long as ten to fourteen days in tap water without food, which shows that the food reserve of the protoplasm may be sufficient to meet its expenditure of energy over this period of time. Cell division stops in a pure line population after the Paramecia have been removed to starvation conditions in tap water for twenty-four hours. Hence it is readily possible to obtain *suspensions of cells which do not divide, that is, where the number of cells remains constant.* It is always well to keep the Paramecia in a comparatively large volume of water before using, for high concentration of suspensions leads sooner or later, depending on conditions, to death of some of the organisms which serve as a food supply for bacteria that may happen to be present and also for other Paramecia which when starved become "hungry" feeders.

Just before using, the washed Paramecia were concentrated at a low speed of the centrifuge so that in 1 cc. of suspension there were

from two thousand to one hundred thousand individuals depending upon the concentration desired for the particular type of experiment. Equal volumes of this suspension were gently withdrawn by use of a 1 or 2 cc. volumetric pipette which had a large smooth opening. Stimulation and injury to individuals by the pipette is often brought about by too rapid suction on a pipette with a small opening and sharp edges. The question arises: Is it possible by this method to obtain equal numbers of Paramecia in different 1 cc. volumes drawn successively by the volumetric pipette? The answer is found by (1) a comparison of the quantities of oxygen absorbed by different 1 cc. samples of Paramecia from the same suspension, and (2) by actual counts of the number of Paramecia in such samples of equal volume. Both of these methods have been used and as will be shown in this and subsequent papers, are trustworthy criteria for determining the number of individuals in unit volume. The error from this source falls within the limits of experimental errors from other sources.

Use of Winkler's method for determining dissolved oxygen. The reagents used for Winkler's method were made up as given by Treadwell and Hall (4) except that 5 cc. instead of 3 cc. of concentrated HCl was used. The thiosulfate solution was standardized at intervals against known weights of freshly resublimed iodine according to Treadwell and Hall (4, p. 645). For simplicity the tables give the oxygen equivalent in cubic centimeters of thiosulfate.

The tap water used for an experiment was kept in carboys and allowed to stand at room temperature. A stream of air was then passed through it for several hours in order that the oxygen in the water might come into equilibrium with that of the air at room temperature. Bottles of equal volumes (137 cc.) were then filled with the tap water from the carboy. The degree of uniformity of oxygen content in such a series of bottles is illustrated by the figures in tables 4, 5 and 6. The variation is on the average less than 0.1 cc. of thiosulfate per 137 cc. volume and where the average of a number of bottles is taken the error in filling of bottles and analysis can be reduced to less than 1 per cent of the oxygen content of 137 cc. of water at atmospheric pressure and a temperature of 20°C.

After filling the bottles 1 or 2 cc. of the concentrated Paramecium suspensions were added and the bottle tightly stoppered. After varying periods of time the water in the bottles containing Paramecia and the blanks without animals were analyzed. A small amount of the liberated iodine is adsorbed by the dead Paramecia. The amount

varies with the number of animals in the bottle and also with the concentration of liberated iodine, but in all cases where the number of Paramecia is not more than five thousand, it is small and practically constant. The error due to adsorption of iodine was eliminated by analyzing at the beginning of the experiment control bottles, one set being blanks without Paramecia and the other containing the same number of Paramecia as was used in the experimental bottles. The difference between averages of these two sets of bottles gave the amount of iodine in cubic centimeters of thiosulfate which was adsorbed by the cells, and in the results are applied as corrections (see tables).

Heilbrunn (5) has pointed out the source of error due to the taking up of iodine by sea urchin eggs and possibly by the secretions liberated by eggs into sea water. This objection does not apply in any measurable degree to this work on Paramecium since, (1) Paramecia do not liberate substances into the tap water which interfere with the analysis, and (2) since the number of Paramecia which are used in each bottle is small in comparison with the number of sea urchin eggs which would utilize an equal quantity of oxygen and (3) the slight loss of iodine that does occur is corrected for as given above. The above method entirely avoids the error which is met with when the water in the bottles containing the eggs or other organisms is siphoned off into a smaller bottle for analysis. Since bottles of equal volume and O_2 content with a practically equal number of cells in each can be obtained, then by computing the average oxygen content of a number of bottles the errors can be reduced to a small value.

When the oxygen consumption of such cells as sea urchin eggs, blood cells or yeast is determined there is no definite index by means of which one can determine when one or more of the cells die and therefore it becomes difficult to know definitely in just what physiological condition the cells are at any particular time. This great experimental disadvantage is practically entirely avoided with Paramecium because one can readily determine by the use of a hand lens, or if necessary under the binocular, when the organisms are abnormal either in shape (approaching cytolysis) or locomotion. Paramecium therefore serves as an ideal organism for accurate quantitative work on intracellular oxidation.

Any experimental procedure which differs from that described above will be given in connection with the particular experiments.

EXPERIMENTAL

Oxygen concentration and the rate of oxygen consumption. Different concentrations of oxygen in tap water were obtained by passing water from the tap through a small heated copper coil into carboys, thereby removing practically all the dissolved air from the water. The water was allowed to cool to room temperature. The desired concentrations of dissolved oxygen were obtained by slowly bubbling compressed oxygen from a tank through the water and analysing samples from time to time in order to determine when the desired concentration of oxygen was obtained. Shaking with air was also convenient. The experi-

TABLE 1

Preliminary experiment. Paramecia left in clear native hay infusion for twenty-four hours, then washed three times in sterile tap water and centrifuged. Volume of bottles 137 cc. Temperature 25±.1°C. 1 cc. thiosulfate = 0.158 cc. O_2 at N. T. P.

BOTTLE	CONTROLS [ANALYZED AT ONCE		ANALYZED AT END OF 6 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	2.00	1.8	1.15		All normal at end of 6 hrs.
2	1.94	1.8			
3	1.94				
Average	1.96	1.8	1.15	*0.65*	
Iodine adsorbed by Paramecia				0.16	
B. High O_2 concentration					
1	13.30	13.00	12.40		Some dead, others dying at end of 6 hrs.
2	13.20	12.90			
3	13.15				
Average	13.21	12.95	12.40	*0.55*	
Iodine adsorbed by Paramecia				0.26	

TABLE 2

Preliminary experiment. Paramecia washed in sterile tap water three times and starved several hours: centrifuged. Volume of each bottle 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2 N. T. P. Temperature 25± .2°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 6 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed in 6 hrs.	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.60	1.58	1.16		All alive and active
2	1.57	1.50			
3	1.70	1.60			
Average	1.62	1.56	1.16	*0.40*	
Iodine adsorbed by Paramecia				0.06	
B. High O_2 concentration					
1	14.6	14.45	14.34		Only a few living at end of 6 hrs. Most of these were injured as shown by movement and shape
2	14.7	14.59	14.35		
3	14.8				
Average	14.7	14.52	14.345	*0.175*	
Iodine adsorbed by Paramecia				0.18	

mental bottles were then immediately filled. The first and the last bottle filled were included in the control blanks in order to detect any perceptible difference in oxygen content of the water drawn first and last.

Tables 1 and 2 are results from two preliminary experiments where the manipulation was not as accurate as in the later experiments. The average amount of oxygen consumed by 1 cc. Paramecia in a bottle during six hours is given in column 4. This is obtained by subtracting the average number of cubic centimeters of thiosulfate per bottle in column 3 from the average number of cubic centimeters per bottle in column 2. It will be noted (table 1) that although the con-

centration of oxygen in one set of bottles, B, was over six times that in the other set, A, nevertheless the average quantity of oxygen consumed in the high oxygen concentration was slightly less than that consumed in the low concentration. The animals in table 1, set A, were all living and normal at the end of six hours. A few of those in B were dead and many were abnormal at the end of six hours. In table 2 where the oxygen concentration in B was about nine times that in A, the oxygen consumed was less than half that consumed in A. This is due to the early death of many of the cells in B caused by the high oxygen concentration. *These results indicate that when the cell is killed by high concentration of oxygen intracellular oxidations stop.*

The remaining experiments given in tables 3 to 6 are an intensive search for any slight effect of oxygen concentration on the rate of intracellular oxidations. The source of error in tables 1 and 2 due to death of cells was carefully eliminated. The results in tables 4, 5 and 6 show the uniformity and accuracy obtainable by Winkler's method with Paramecium in tap water.

In order to detect any slight effect of concentration of oxygen on the rate of intracellular oxidation which might occur, two types of experiments were tried. The first type is represented in table 3 and consisted in determining the time rate of oxygen absorption beginning at a concentration which is near the lower limits of the oxygen concentration in which Paramecium can survive. The cells were allowed to begin the consumption of a quantity of oxygen equivalent to an average of 1.476 cc. thiosulfate per 137 cc. volume. The amounts of oxygen consumed in successive periods of time were determined in order to see if the amount of oxygen consumed per hour differed as the concentration became less. The results are given in table 3 and are shown in the curve, figure 1. At the end of seven and one-half hours the first Paramecia in the bottles were just beginning to die. Hence the results up to and including the first seven and one-half hours are free from sources of error due to death. During the period from seven and one-half to ten hours an increasing number of deaths took place but the proportion of dead to living cells was small hence the decrease in the amounts of oxygen absorbed due to death is not noticeable from the figures. With the exception of the first four hour period, the oxygen consumed per hour is practically the same even down to the concentration which is unable to support the life of the cell. During the first four hour period slightly more oxygen was absorbed per hour than in the period from four to seven and one-half hours. This, as was

discovered later, is very probably due to the progressive difference in the nutrition of the cells under conditions of starvation. This point will be dealt with in a subsequent paper. From this experiment the rate of intracellular oxidation seems to be quite independent of the concentration of oxygen, even at very low concentrations, yet the cell is unable to absorb the oxygen (at a sufficiently rapid rate?) below a certain minimal concentration.

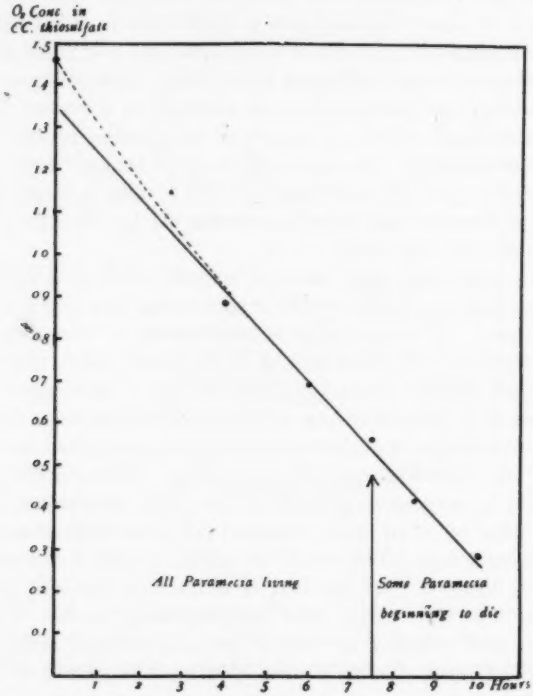

Fig. 1

It is evident that this minimal oxygen concentration varies for different individual cells, for most of the Paramecia were still normal and active at the end of ten hours or at a concentration equivalent to 0.41 cc. thiosulfate per 137 cc. of water, while others died at a concentration equivalent to 0.56 cc. of thiosulfate per 137 cc. of water. Evidently different cells differ in their ability to absorb oxygen at certain

low concentrations. What factors determine what this minimal concentration shall be for each cell?

The second type of experiment was like that used in tables 1 and 2 except that a larger number of bottles was used and the error due to death of Paramecia was carefully avoided by stopping the experiment before or when first signs of injury or death of cells were noticed. Greater care was also employed in manipulation and preparation of the Paramecia. Table 4 gives the results of an experiment lasting

TABLE 3

Showing the rate of oxygen consumption by Paramecium in low oxygen concentration. Paramecia not starved but removed directly to sterile tap water, washed three times and then used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 25± .1°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 4 HRS.		ANALYZED AT END OF 6 HRS.		ANALYZED AT END OF 7.5 HRS.		ANALYZED AT END OF 8.5 HRS.		ANALYZED AT END OF 10 HRS.	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed	1 cc. Paramecia added	O_2 consumed
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	1.48	1.45	0.90		0.72		0.60		0.40		0.27	
2	1.40	1.45	0.86		0.68		0.55		0.46		0.23	
3	1.55	1.47	0.89		0.65		0.57		0.44		0.32	
4			0.90		0.72		0.53		0.37		0.33	
Average.....	1.476	1.456	0.887	*0.569*	0.692	*0.764*	0.563	*0.894*	0.417	*1.039*	0.287	*1.169*
Iodine adsorbed by Paramecia.		0.02										

two hours and thirty-five minutes. At the end of this time the first signs of abnormal movement occurred in those animals in high concentration of oxygen. The difference in the quantitites of oxygen used by the two sets of cells lies within the limits of error of the experiment.

In table 5 are given results of an experiment which differs chiefly from that of table 4 in that the duration of the experiment was much longer (thirteen hours). The oxygen concentrations also only differ by an amount which is often met with by the organisms in nature.

Semi-starvation of the animals previous to the experiment took place in clear native medium for twenty-four hours. They were then washed and starved for a second period of twenty-four hours in tap water, before using. The protoplasm of the cells was clear. They had

TABLE 4

Paramecia starved fifteen hours in sterile water, then washed three times in sterile tap water before using. Volume of bottles 137 cc. 1 cc. thiosulfate= 0.158 cc. O_2. Temperature 25 ± .1°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 2 HRS. 35 MIN.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	2.68	2.65	2.50		All perfectly normal and active at end of 2 hrs. and 35 min.
2	2.76	2.65	2.42		
3	2.73	2.65	2.38		
4	2.75	2.70	2.45		
5			2.40		
6					
Average.....	2.75	2.66	2.42	*0.24*	
Iodine adsorbed...				0.09	
B. High O_2 concentration					
1	11.50	11.35	11.25		At 2 hrs., 35 min. many swimming slowly, a few deformed but none were dead
2	11.65	11.40	11.14		
3	11.55	11.45	11.25		
4	11.70	11.45	11.05		
5			11.10		
Average.....	11.60	11.41	11.16	*0.25*	
Iodine adsorbed...				0.19	

therefore entered upon a stage of "acute" starvation unlike those in the experiment of table 3. The oxygen consumption was independent of the concentration of oxygen. This experiment was repeated with the same results. It should be noticed in this experiment where the

animals were severely starved, that all the Paramecia in low oxygen concentration were still alive at an average concentration of 0.13 cc. thiosulfate per 137 cc. water, while in the experiment in table 3 where the animals had not been starved previous to the experiment, they

TABLE 5

Paramecia partly starved for twenty-four hours in clear native medium, then for twenty-fours in tap water, washed twice then used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 25 ± .2°C.

BOTTLE	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 13 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.57	1.55	0.10		All perfectly normal at end of 13 hrs. None dead.
2	1.58	1.50	0.20		
3	1.65	1.60	0.10		
4			0.10		
5			0.15		
Average.....	1.60	1.55	0.13	*1.42*	
Iodine adsorbed...				0.05	
B. High O_2 concentration					
1	4.40	4.35	3.01		All perfectly normal at end of 13 hrs. None dead.
2	4.45	4.40	2.99		
3	4.45	4.35	3.02		
4			2.93		
5			2.90		
Average.....	4.43	4.366	2.97	*1.396*	
Iodine adsorbed...				0.064	

began to die at a concentration of oxygen equivalent to about 0.56 cc. of thiosulfate per 137 cc. This suggests that the nutritive condition of the cells is one of the factors which determines the minimal concentration of oxygen at which oxidations in the cell and life can proceed.

All of the previous experiments were carried out at a temperature

of 25 ± .1 or .2°C. It was thought that if the temperature was lowered and the duration of the experiment was increased that possibly any slight effects of differing oxygen concentration on the rate of oxidations might appear. Table 6 gives the results of an experiment carried on

TABLE 6

Paramecia starved twenty-four hours in diluted native medium. Washed three times in sterile tap water before using. A very concentrated suspension of Paramecia was used. Volume of bottles 137 cc. 1 cc. thiosulfate = 0.158 cc. O_2. Temperature 13.5 ± .2°C.

BOTTLES	CONTROLS ANALYZED AT ONCE		ANALYZED AT END OF 20 HRS.		REMARKS
	1	2	3	4	
	Blanks	1 cc. Paramecia added	1 cc. Paramecia added	O_2 consumed	
A. Low O_2 concentration					
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	1.80	1.65	0.80		All alive and active at end of 20 hrs.
2	1.80	1.65	0.87		
3	1.85	1.60	0.87		
4		1.67	0.90		
5		1.65	0.85		
Average.....	1.81	1.66	0.86	*0.80*	
Iodine adsorbed...				0.15	
B. High O_2 concentration					
1	7.10	6.70	5.90		All alive and active at end of 20 hrs.
2	7.05	6.85	5.95		
3	7.00	6.80	5.90		
4		6.80	6.00		
5			6.00		
Average.....	7.05	6.79	5.95	*0.84*	
Iodine adsorbed...				0.26	

at 13.5°C. In this experiment there were at least three times as many Paramecia in 1 cc. as in any of the previous experiments; this accounts for the large amount of iodine adsorbed by the Paramecia. The difference in the average amounts of oxygen consumed in twenty

hours was 0.04 cc. thiosulfate. This lies within the limits of possible error for the experiment so that no evidence was found for an effect of oxygen concentration on oxidations at this low temperature.

Concentration of oxygen and rate of intracellular oxidations in other animals. It has generally been agreed among physiologists, on the basis of results from experiments on mammals, that the rate of intracellular oxidations is widely independent of the concentration of oxygen in the medium surrounding the cells. These animals are obviously poorly adapted for experimentation where accurate quantitative data on such questions as the rate of oxidations in the cell under normal conditions are desired.

In experiments on certain invertebrates Thunberg (6) found that by placing Lumbricus in 96 per cent oxygen the oxygen consumption was 44 per cent greater than that in air. For Limax similar results were obtained, and by lowering the concentration of oxygen the rate of oxygen consumption by Tenebrio and Limax decreased. Later Henze (7) found that the quantity of oxygen absorbed per hour by the coelenterates Actinia, and Anemonia, and the worm Sipunculus was influenced very largely by the concentration of dissolved oxygen. In other animals such as the crustacean Carcinus, the molluscs Aplysia and Eledone and the bony fishes Coris and Sargus, the rate of oxygen consumption was quite independent of the concentration of oxygen. Thunberg interpreted his results to mean that the rate of intracellular oxidations was determined by the mass of the reacting substances according to the mass law, when the oxygen concentration was below a certain value. Above this concentration of oxygen no increase in oxygen consumption was to be expected because for all practical purposes it could be considered infinite in amount and therefore had the same relation to the reaction velocity and equilibrium conditions of the oxidation reaction as the concentration of water has to the reaction velocity and equilibrium in, for example, the inversion of cane sugar.

Henze interprets his results in essentially the same way as Thunberg does but suggests that in cells where the actual rate of oxygen consumption due to oxygen deficiency is below the maximum, anaorobic processes supplement the aerobic processes and as a result the cells do not die because of too low oxygen concentration. To sum up, the results so far, on lower animals indicate that the relation between the concentration of oxygen and the rate of intracellular oxidations differs in different animals, in particular with respect to the oxygen concen-

tration at which an effect on the oxidative processes is noticeable. Much greater accuracy in the methods for quantitative studies on intracellular oxidation in multicellular animals is necessary before a satisfactory statement in detail of the conditions for these forms can be made.

SUMMARY

1. Paramecium serves as an ideal organism for accurate quantitative studies on intracellular oxidations.

2. In Paramecium the oxidations stop when the cell is killed by too high oxygen concentration.

3. In a pure line of Paramecium from the same culture different cells differ in respect to the minimal oxygen concentration in which they can continue to live.

4. The rate of intracellular oxidation in Paramecium is independent of the concentration of oxygen. The concentration of oxygen may vary from a minimum of about 0.04 cc. of O_2 at N.T.P. per 137 cc. to 2.2 cc. O_2 at N.T.P. per 137 cc. or fifty-five times the minimal concentration, without affecting the rate of oxidations. This is true for a temperature of 13.5° as well as 25°C.

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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

II. The Rate of Oxidations in Paramecium caudatum and its Independence of the Toxic Action of KNC

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Most of the recent work on the problem of the nature of the effects of anesthetics and narcotics on cells seems quite definitely to support the conclusion that these substances do not exert their distinctive effects upon cells by primarily inhibiting intracellular oxidations. Where a lowered rate of oxygen consumption or carbon dioxide production as the result of injection or immersion in solutions of alcohol, ether, chloral hydrate and the urethanes has been observed, it can usually be interpreted as an indirect effect, due for example to lowered muscular tone or death of cells (1), (2), (3), (4).

In striking contrast to these experimental results stand those from the cyanides. It is very generally agreed that the toxic action of the latter is due to their specific power of inhibiting intracellular oxidations. Any cell which can be isolated and subjected to direct observation of its structure and visible processes under experimental conditions has often, for obvious reasons, many advantages over complex cell aggregates; consequently some of the most direct and convincing evidence for this specific inhibitory action by the cyanides has been obtained from experiments on sea urchin eggs (5), (6).

On the basis of this and other evidence for specific inhibitory action by cyanides on intracellular oxidations, hypotheses and conclusions have often been arrived at by others which if proven to be correct are of importance. Potassium cyanide has been used in studies on the action of the respiratory center in mammals, its effects being attributed to its inhibitory action on the oxidations in the nerve cells of the respiratory center (7). Child (8) and his colleagues have made extensive use of the susceptibility of organisms to the toxic action of the cyanides

in studies on morphogenesis. They assume that the rate of oxidations serves as the most satisfactory available measure of the rate of (total?) metabolism, and that the rate of metabolism determines the course of morphogenetic processes, such for example as localization of body axis in regenerating pieces of planaria and various coelenterates. If the cyanides do have a specific inhibitory effect on oxidations in general they naturally offer interesting possibilities for experiment.

The purpose of the present paper is to show that in Paramecium caudatum the rate of intracellular oxidations is entirely independent of the action of KNC even in concentrations which injure and finally kill the cell by cytolysis.

RATE OF OXYGEN CONSUMPTION BY PARAMECIUM IN SOLUTIONS OF KNC IN TAP WATER

The procedure for oxygen determination and preparation of Paramecia has been described in a previous paper (9). Table 1 gives the results of a preliminary experiment carried out before the methods described in the first paper of this series had been fully worked out, therefore several sources of error such as that in the filling of the bottles, adsorption of iodine and the drawing of samples of Paramecia from the suspension were not eliminated as fully as in the remaining three experiments. It is given because in spite of some non-uniformity in oxygen concentration in the bottles the results show very clearly that KNC does not inhibit the oxidations to any noticeable degree even in the concentrations which killed some or nearly all of the Paramecia.

It will be seen from column 7 that in those bottles where a large number of Paramecia were dead at the end of fifty-one hours, the total oxygen consumed was in general a little less than in the bottles where all the Paramecia were living. The average quantity of oxygen consumed which is equivalent to 2.32 cc. thiosulfate per 150 cc., was practically the same in the bottles containing Paramecia without KNC (column 4), as in those bottles indicated by the bracket in column 7 where it was equivalent to an average of 2.43 cc. thiosulfate per 150 cc. The lower average oxygen consumption in column 7 is entirely accounted for by the death of Paramecia which occurred during the experiment in the higher concentrations of KNC. In spite of the evident errors due to non-uniformity in conditions for each bottle the fact is clear, from a glance at the figures in columns 2, 4 and 7, that intracellular oxidations were not inhibited to any marked degree by

the cyanide either in the weak or in the strong concentrations, and that an average of almost 3.00 cc. thiosulfate equivalent of oxygen was con-

TABLE 1

Preliminary experiment. Bottles were filled from tap by rubber tube. The Paramecia were centrifuged, washed twice in tap water and used immediately without starving. The KNC solution was first added, then 1 cc. Paramecium suspension was added and the bottle stoppered and shaken. Temperature 21 ± 2°C.

BOTTLE	CONTROLS NO KNC ADDED				KNC ADDED AND 1 CC. PARAMECIA. ANALYZED AT END OF 51 HOURS			CONDITION OF PARAMECIA AT END OF 51 HOURS
	1 cc. Paramecia added. Analyzed at once		1 cc. Paramecia added. Analyzed at end of 51 hours					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	Volume of bottle	O_2	Volume of bottle	O_2	N/10 KNC	Volume of bottle	O_2	
	cc.	*cc. thio*	*cc.*	*cc. thio*	*cc.*	*cc.*	*cc. thio*	
1	155	6.05	160	3.05	3.0	151	3.55	Nearly all dead
2	157	5.75	137	2.02	2.5	149	3.43	About ¼ living
3	147	5.05	163	3.00	2.2	143	3.50	About ⅓ living
4	139	5.30	160	2.50	2.0	149	3.20	About ½ living
5	153	6.25	146	2.40	1.8	142	2.27	More than ½ alive
6	154	5.82	156	2.10	1.6	160	3.70	More than ½ alive
7			145	1.85	1.4	154	2.35	Some dead
8			142	1.75	1.2	150	2.40	Very few dead
9			149	2.23	1.0	155	2.45	
10			157	2.45	0.8	153	2.70	
11			139	2.05	0.6	154	.75	
12			153	2.40	0.4	152	2.55	None dead in any of these bottles
13			164	2.90	0.2	150	2.65	
14			156	2.32	0.2	147	2.15	
15					0.1	166	2.65	
16					0.05	138	1.80	
Average cubic centimeters of thio. per 150 cc.		*5.67*		*2.32*			*2.74*	

sumed by the Paramecia in the KNC solutions. Is the rate of oxidations in Paramecium completely unaffected by cyanide? For an answer to this question greater degree of accuracy is necessary.

In the following experiments the bottles were of equal volume and contained nearly equal amounts of oxygen, the degree of accuracy is greatest in experiments the results of which are given in tables 2 and 3. Controls for determining the average amount of iodine adsorbed by Paramecia, are given in columns 1 and 2. This is equivalent to 0.43 cc. thiosulfate, a relatively large amount due to the large number of Paramecia in each bottle (table 2). The Paramecia in bottles containing 3 and 2.6 cc. N/10 KNC would probably not have lived more than a few hours longer, for a number in each bottle were dead and many were deformed and showed abnormal swimming movements. In bottles containing less than 1.4 cc. M/10 KNC the cells were perfectly normal and active and would undoubtedly have lived much longer. In other experiments similar to this one the Paramecia lived in concentrations of 0.05 cc. up to 0.4 cc. N/10 KNC in 137 cc. tap water for several days, until nearly all the oxygen was consumed or death occurred as a result of starvation. The average amount of oxygen in cubic centimeters of thiosulfate remaining after sixteen and one-half hours in the first six bottles of column 5 is 4.42. That of the last six bottles is 4.54 cc. thiosulfate. No animals had died in any of the bottles although the concentrations of KNC in the first three bottles were sufficient to kill many of the Paramecia had they been left for twenty-eight hours as shown in column 7. Evidently a lethal concentration of KNC in this case accelerated the oxidations or else was entirely without effect. The first three bottles (column 7) show a slightly lower oxygen content than the remaining ones. The only way I can account for this is by an accelerating effect or increased oxygen consumption due to stimulation of the organisms by the cyanide or more probably by errors in filling bottles.

A final experiment (table 3) was performed in which the greatest care was taken to avoid errors in manipulation. The range of variation of the cyanide concentrations—5 cc. to 0.05 cc. N/10 KNC—was greater than in previous experiments. It happened that the resistance to KNC of this lot of pure line Paramecia was greater than that of those used in any of the previous experiments so that at the end of twenty-nine and one-half hours relatively very few Paramecia were dead, even in the bottle containing 5 cc. N/10 KNC. Results of an analysis of the conditions for variability in resistance to KNC by Paramecium will be given elsewhere.

At the end of ten hours all Paramecia were alive in all the bottles. Those in the bottles containing 5 cc. KNC were moving more slowly

than the others and a few were abnormal in shape, an indication that death was approaching. At twenty-nine and one-half hours a few were

TABLE 2

Paramecia starved in clear native medium for 48 hours, centrifuged, washed in clear tap water twice, then used. All bottles had a volume of 137 cc. Temperature 16°C.

BOTTLE	CONTROLS ANALYZED AT ONCE			ANALYZED AT END OF 16½ HOURS		ANALYZED AT END OF 28 HOURS		REMARKS
		1 cc. Paramecia added		1 cc. Paramecia added		1 cc. Paramecia added		
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	Blanks	N/10 KNC	O_2	N/10 KNC	O_2	N/10 KNC	O_2	All living at 16½ hours
	cc. thio	*cc.*	*cc. thio*	*cc.*	*cc. thio*	*cc.*	*cc. thio*	
1	5.9	3.0	5.45	3.0	4.18	3.0	3.96	At 28 hours few dead, many deformed
2	5.5	2.0	5.05	2.6	4.35	2.6	3.80	At 28 hours few dead, some sluggish
3	5.7	1.0	5.30	2.2	4.55	2.2		
4	5.7	0.8	5.35	1.8	4.55	1.8	3.90	All had nearly normal shape and were active
5	5.7	0.6	5.15	1.4	4.44	1.4	4.07	All normal and active
6		0.2	5.35	1.2	4.46	1.2	4.13	All normal and active
7				1.0	4.70	1.0	4.10	All normal and active
8				0.8	4.45	0.8	4.22	All normal and active
9				0.6	4.70	0.6	4.50	All normal and active
10				0.4	4.50	0.4	4.12	All normal and active
11				0.2	4.37	0.2	4.20	All normal and active
12				0.1	4.55	0.1	4.50	All normal and active
13				0.05		0.05	4.25	All normal and active
Average	5.70		5.27		4.48		4.14	
Iodine adsorbed			0.43					
Average O_2 consumed					*0.79*		*1.13*	

dead in the remaining bottle containing 5 cc. KNC. Blisters were present in many and movement was slow. In 4 cc. KNC a very few were dead, many moved slowly. In the remaining bottles there was

an increasing number of normal Paramecia until in the bottle containing 3 cc. KNC no injurious effect on form or movement could be seen. One cubic centimeter of Paramecium suspension contained relatively few individuals in this experiment as shown by the small amount of iodine adsorbed which was equivalent to only 0.13 cc. thiosulfate.

TABLE 3

Paramecia starved in native medium for 24 hours, washed twice and starved in tap water for 12 hours, then used. All bottles 137 cc. Temperature 22°C.

BOTTLE	CONTROLS KNC ADDED ANALYZED AT ONCE			ANALYZED AT END OF 10 HOURS		ANALYZED AT END OF 29½ HOURS		REMARKS
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	
	KNC N/10	Blanks O_2	1 cc. Paramecia added O_2	KNC N/10	1 cc. Paramecia added O_2	KNC N/10	1 cc. Paramecia added O_2	At 10 hours all were living
	cc.	*cc. thio*	*cc. thio*	*cc.*	*cc. thio*	*cc.*	*cc. thio*	
1	5.0	5.40	5.35	5.0	4.22	5.0	3.40	29½ hours a few dead
2	4.5	5.45	5.20	4.5	4.20	4.5	3.20	
3	4.0	5.40	5.20	4.0	4.35	4.0	3.30	29½ hours, all living
4	3.5	5.35	5.15	3.5	4.33	3.5	3.20	
5	3.0		5.30	3.0	4.32	3.0	3.30	29½ hours, all living
6	2.5		5.30	2.5	4.30	2.5	3.14	29½ hours, all living
7	2.0		5.25	2.0	4.40	2.0	3.20	29½ hours, all living
8	1.0	5.40	5.35	1.0	4.30	1.0	3.40	29½ hours, all living
9	0.5	5.30	5.20	0.5	4.35	0.5	3.10	29½ hours, all living
10	0.05		5.20	0.05	4.25	0.05	3.20	29½ hours, all living
Average.....		*5.38*	*5.25*		*4.30*		*3.24*	
O_2 consumed...					0.95		2.01	

As a further control for this experiment the average amount of oxygen per bottle absorbed by the same number of Paramecia in tap water *without* cyanide was determined and found to be the same as the average amount of oxygen absorbed by the Paramecia in the cyanide solutions. This control was a part of another experiment carried out for a different purpose but with the same Paramecium suspension and at the same time as the experiment in table 3. It is not included in the table.

The foregoing experiments therefore demonstrate beyond doubt that the rate of oxidations is entirely independent of the toxic action of the cyanide. They further indicate that after cytolysis has occurred no more oxygen is used up. To further test this interesting point the following experiment was carried out.

Sixteen bottles of equal volume (137 cc.) were filled with tap water and divided into four sets, indicated below in table 4 by A, B, C and D. One cubic centimeter of Paramecium suspension was then added to each bottle and immediately thereafter the quantities of cyanide given

TABLE 4

Same Paramecium suspension as used in experiment in table 3. Volume of bottles 137 cc.; M/1 KNC was used. Temperature 21°C.

BOTTLE	CONTROLS				ANALYZED AT END OF 25½ HOURS 1 CC. PARAMECIA ADDED		REMARKS	
	Anaylzed at once			Analyzed at end of 25½ hours 1 cc. Paramecia added, no KNC				
	1 cc. Paramecia added, no KNC	1 cc. Paramecia added			M/1 KNC added	O_2	20 minutes after beginning experiment	4½ hours after beginning experiment
		M/1 KNC added	O_2					
	A		B	C		D		
	cc. thio	*cc.*	*cc. thio*	*cc. thio*	*cc.*	*cc. thio*		
1	5.35	5	5.30	3.9	5	5.20	All dead	All dead
2	5.30	4	5.25	3.80	4	5.70(?)	Most dead	All dead
3	5.30	3	5.38	3.80	3	5.08	Many dead	All dead
4	5.38	2	5.12	3.90	2	4.60	None dead	Many dead
Average....	5.33		5.26	3.85		5.39		

in the table were added to the bottles of sets B and D. The concentration of the KNC solution was N/1 or ten times greater than that used in the previous experiments, in order that the Paramecia might be killed quickly. Any oxygen consumed by the cytolysed cells could then be detected. Sets A and B were analyzed at once, serving as controls. Sets C and D were analyzed at the end of twenty-five and one-half hours. Records of the death rate of the animals were taken at frequent intervals.

All the Paramecia in all the bottles containing 5 and 4 cc. KNC were dead at the end of fifty minutes. All the Paramecia in the remaining bottles died within eight hours from the beginning of the experiment.

The oxygen content at the end of twenty-five and one-half hours, in

the bottles containing 3 and 2 cc. KNC shows that some oxygen had been consumed; this is to be expected since many of the Paramecia remained alive for from one hour in the bottle containing 3 cc. KNC to about seven hours in the bottle containing 2 cc. KNC. This experiment with the previous ones clearly shows that the oxidations stop when the cell is killed by KNC.

Does KNC inhibit oxidations which might go on in cytolysed cells of Paramecium in the absence of KNC? The answer is that there are in all probability after cytolysis of the cell, no oxidations to be inhibited by KNC, for it has been shown by Warburg (6) that when fertilized sea urchin eggs are killed by mechanical disintegration the oxidations practically cease. It was further shown in a previous paper (9) that intracellular oxidations in Paramecium stop when the cell is killed by too high oxygen concentration. In other words it appears that the oxidations stop regardless of the methods used for bringing about cytolysis. Warburg (6) attributed this disappearance of oxidations in fertilized sea urchin eggs to disappearance of the structure of the protoplasm during cytolysis. In other words that maintenance of the structure of protoplasm was a necessary condition for continued intracellular oxidations. There are reasons for believing that the conditions for intracellular oxidations which are related to the ordinary respiratory process in cells, for example in the muscle cell, are different from the conditions in such types of oxidations as occur in extracts of cells, for example the oxidation of tyrosin in potato juice. For the muscle cell the observations of Fletcher and Hopkins (10) indicate that the oxidation of or more probably the oxidation leading to replacement of lactic acid is conditioned by the existence of a normal cell structure, and that when the cells are mechanically disintegrated the conditions for oxidation or replacement of lactic acid are largely removed. The oxidation of tyrosin by means of the oxidase tyrosinase continues in aqueous extracts of the cells of various fungi (11). These and other facts indicate that biological oxidations of carbohydrates and lipoids and their physiological derivatives which are concerned with transformation of energy from oxidations into mechanical work may occur under quite different conditions than those which occur in solutions of tissue extracts. Perhaps one should therefore not wonder that enzymes which facilitate oxidations of sugars and fats in presence of free oxygen have not been found. Further work on these questions is necessary before any satisfactory conclusions can be drawn.

SUMMARY

1. The rate of intracellular oxidations in Paramecium caudatum is entirely independent of the toxic action of KNC.

2. Inferences based on supposed similarity of action by KNC on different cells in respect to intracellular oxidation are not strictly permissible unless it is shown by direct measurement on each type of cell, or in some other way, that KNC inhibits the oxidations.

3. Intracellular oxidations stop when Paramecium undergoes cytolysis in KNC solutions. But this stopping of the oxidations is not necessarily correlated with the toxic action of KNC for oxidations also stop when the cell is killed by oxygen in too high concentrations.

4. If no. 3 is true for other cells, then a necessary condition for proof that cyanides inhibit oxidations is that the cells treated with cyanide must be shown not to be cytolysed by the cyanide.

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A QUANTITATIVE STUDY OF THE EFFECT OF RADIUM RADIATIONS UPON THE FERTILIZATION MEMBRANE OF NEREIS

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Existing studies of the effects of radium radiations[1] upon protoplasm fail to yield quantitative data of sufficient accuracy to form a basis for an investigation of the nature of the processes involved. A search has consequently been made for a physiological reaction to these radiations which could be measured with precision. A membrane is formed about the egg of the marine worm, Nereis limbata, when it is fertilized. If the egg has been exposed to the radiations from radium before fertilization occurs, the membrane will be abnormally thick when it is formed. The volume of this structure is a function of the amount of radiation which the eggs have received. Because the magnitude of this change is great—several hundred per cent—and because it may be produced by relatively small quantities of radiation a very precise and convenient test object is available, which may prove of value in solving many problems in the physiology of radioactivity.

The cortical changes which accompany fertilization of this egg have been described by Lillie (1). Upon fertilization the *vitelline membrane* of the unfertilized egg becomes separated from the underlying protoplasm by a layer of fluid, the *perivitelline space*. It cannot be stated at present which of these structures is altered by radiation. The word membrane is used in this paper to denote the optically homogeneous, colorless layer limited by the outer surface of the egg and by the "granular," yolk-laden protoplasm.

The increase in volume of the membrane is not accompanied by any diminution in the volume of the "granular" protoplasm as the meas-

[1] We are indebted to Dr. William Duane for placing a supply of radium belonging to the Cancer Commission of Harvard University at our disposal.

urements in table 1 indicate. The changes produced by radiation cannot be considered to depend on the *secretion* of an unusual quantity of material from the egg. Rather it is due to the absorption of an abnormal amount of sea water, or some of its components.[2]

The swelling of the membrane of radiated eggs takes place gradually during a considerable interval of time. A consideration of figure 1 will show that the rate of swelling decreases as the process progresses. By waiting until the process is approaching completion errors due to slight variations in the time of measurement are minimized and the percentage error in observation decreased. On the other hand eggs which have cleaved cannot be measured with precision because of the unequal thickness of different parts of the same membrane. It was

TABLE 1

September 11, 1917. Nereis eggs exposed to radium emanation for fifteen minutes

NUMBER OF EGGS MEASURED	INTENSITY	MEAN TOTAL DIAMETER	MEAN THICKNESS OF MEMBRANE	MEAN DIAMETER OF GRANULAR PROTOPLASMS
	millicurie centimeters	μ	μ	μ
12	0	131.5	3.5	124.5
11	9.0	147.5	9.0	130.0
11	22.5	152.0	10.7	130.5
11	100.8	156.0	14.0	127.0

found advisable to measure the membranes fifty or sixty minutes after fertilization during the warmer parts of the summer; as much as an hour and a half could be allowed to elapse in cooler weather. In every case each lot of eggs was measured after the same period in any single experiment.

The influence of the time elapsing between radiation and fertilization upon the thickness of the membrane has been determined. Several lots of eggs from a single female were exposed to a glass tube 12 mm. long containing 37.5 millicuries of radium emanation at a distance

[2] The phenomenon here described should not be confused with the observation of Packard (2) that some radiated eggs are larger than unradiated eggs. This condition which apparently occurs only when the egg is radiated more severely than in the present experiments, is due to a failure of the egg to secrete its jelly layer at once. I have not observed any alteration of the jelly secretion at the doses considered in this paper. Packard's phenomenon differs also from the swelling of the membrane in persisting only eighty minutes after fertilization. The change in the membrane is apparently irreversible.

of 7 mm. for ten minutes. One lot of these eggs was fertilized at once and other portions fertilized after increasing intervals of time. Measurements were made of the thickness of the membranes of eggs from

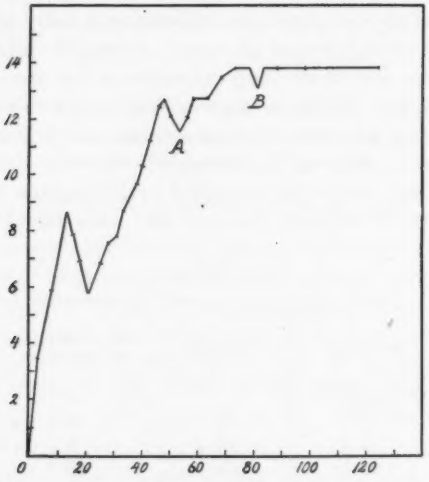

Fig. 1. Process of swelling of the membrane of a single Nereis egg. Time elapsing after fertilization measured in minutes along the abscissa. Thickness of membrane measured in micra along the ordinate. At *A* polar bodies were formed. At *B* the first cleavage occurred.

TABLE 2

MINUTES BETWEEN RADIATION AND FERTILIZATION	NUMBER OF EGGS MEASURED	MEAN THICKNESS OF MEMBRANE IN MICRA
0	10	8.5 ± 0.4
16	10	7.3 ± 0.4
30	11	7.6 ± 0.3
45	12	6.1 ± 0.5
60	12	9.5 ± 0.2
75	10	8.0 ± 0.2
90	10	7.9 ± 0.3
Unradiated control		3.1 ± 0.1

each portion between fifty and sixty minutes after fertilization. In table 2 the average measurement for each lot of eggs is tabulated. No significant recovery of the eggs occurred. Within the time limits

of the experiment the change produced by radiation is irreversible. Consequently the time which elapses between radiation and fertilization was disregarded in subsequent experiments.

Measurements were made according to the following routine. A female Nereis which had been caught the preceding night was cut open in a dry watch glass. A few drops of eggs were placed under the tube of radium emanation for the desired interval. At a convenient time thereafter—in practice never more than fifteen minutes later—the eggs were fertilized and placed in 8 or 10 cc. of sea water. At a definite time (depending upon the temperature) after fertilization a few eggs were placed in a hollow ground slide, of sufficient depth to prevent compression by the cover glass, and measured with the aid of a

TABLE 3

September 13, 1917. Nereis eggs exposed in successive lots to 34.9 millicuries of radium emanation in tube 10.2 mm. long, 6.3 mm. distant, for ten minutes (564 millicurie centimeter minutes). Measured ninety to one hundred minutes after fertilization

NUMBER OF EGGS MEASURED	MEAN VOLUME OF MEMBRANES $10^3\mu^3$
13	7.05 ± 0.18
19	6.85 ± 0.30
18	6.70 ± 1.80
18	6.85 ± .46
19	7.05 ± 0.43
18	6.85 ± 0.55

high power objective and ocular micrometer. One egg after another was measured as rapidly as possible during five or ten minutes. Ten to twenty-five eggs from each lot were measured, their average taken and from these figures the volume of the membrane was computed in cubic micra, taking the diameter of the granular protoplasm to be 128 μ. In practice it was found possible to measure the membrane—which varied under the conditions of experiment from 0.8 to 7.0 divisions of the ocular scale (2.2 μ to 20 μ) to within 0.2 of a division of the scale (0.56 μ). Examination of the data will show that the probable error of the mean for a series of measurements rarely exceeds 5 per cent of the whole. In order to test practically the importance of uncontrollable sources of variation several lots of eggs from a single female were radiated successively with the same tube of radium, at the

same distance and for the same time. The dosage was selected so as to produce a membrane of such a magnitude that slight differences in treatment could be most readily detected. Measurements were then made of each lot of eggs at a uniform time after fertilization. The values obtained are recorded in table 3. It is clear that variation due to uncontrollable causes is very slight indeed.

The relation between the quantity of radiation and the amount of effect produced upon protoplasm is of the greatest importance, not only because of its obvious bearing upon the question of dosage in radiotherapy, but because its establishment will enable many theoretical and practical problems in the physiology of radioactivity to be attacked. The quantity of rays falling upon a given area from a radioactive source depends upon the intensity of radioactivity and the time during which it acts. Intensity in turn depends upon the quantity of radioactive substance present, and its distance from the area under consideration. In the present paper a notation derived by Dr. Alexander Forbes from the formula of Wood and Prime (3) will be employed to express the quantity of radiation. As a standard the intensity of rays emitted by 1 millicurie of radium emanation (1 mgm. element) located at a *point* at a distance of 1 cm. is taken as the *intensity unit* and designated 1 millicurie centimeter. The quantity of radiation emitted by 1 millicurie centimeter in one minute is taken as the *quantity unit* and designated 1 millicurie centimeter minute. Under actual conditions the radium emanation is not confined to a point but is distributed through the length of a slender glass tube. Consequently the intensity does not vary inversely with the square of the distance, but according to the formula

$$I = \frac{Q \cdot \theta}{a \cdot b}$$

when I = intensity in millicurie centimeters
Q = quantity of the radium emanation in millicuries
θ = the angle measured in radians between a perpendicular line drawn from the midpoint of the tube to the point under consideration, (i.e., the radiated cell) and a line drawn from the end of the tube to the same point.
a = perpendicular distance in centimeters from tube to point under consideration.
b = one-half length of tube in centimeters.

It follows that the quantity of radiation is expressed by

$$I \cdot t \quad \text{or} \quad \frac{Q\,\theta \cdot t}{a \cdot b}$$

The variables to be studied then are intensity and time.[3]

A series of experiments was performed to determine the relation between the degree of swelling of the membrane of the fertilized Nereis

TABLE 4

September 5, 1917. Nereis eggs radiated for various periods at an intensity of 29.1 millicurie centimeters. Measured sixty to sixty-five minutes after fertilization

$A = -1.6 \qquad c = 8.12$

NUMBER OF EGGS MEASURED	TIME OF RADIATION	VOLUME OF MEMBRANE OBSERVED $10^3\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^3\mu^3$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	minutes			
11	6	5.19 ± 0.18	4.72	+0.47
12	10.5	7.13 ± 0.19	6.70	+0.43
12	17.5	8.89 ± 0.19	8.50	+0.39
11	21.5	8.80 ± 0.19	9.22	−0.42
11	26.0	9.75 ± 0.25	9.89	−0.14
11	31.0	11.10 ± 0.14	10.52	+0.58
12	36.0	10.54 ± 0.38	11.03	−0.49
12	41.0	11.55 ± 0.13	11.50	+0.05
12	46.0	12.09 ± 0.34	11.90	+0.19
12	52.0	12.05 ± 0.27	12.32	−0.27
12	56.0	13.80 ± 0.86	12.60	+1.20
12	60.0	12.40 ± 0.35	12.82	−0.42
6	0	1.60 ± 0.06		

egg and the length of radiation: the intensity being kept constant. Several drops of eggs from a female Nereis were placed under a tube 12 mm. long containing 13.24 mc. radium emanation at a distance of 6 mm. At intervals a small drop of eggs was removed, fertilized and measured. Table 4 indicates the result.

A consideration of these data shows that as the period of radiation is increased the volume of the resulting membrane is also increased.

[3] The glass tube was sufficiently thick to absorb the alpha rays. The effects are due consequently to the beta and gamma rays. The distance between the tube and the tissue never exceeded 2 cm. The absorption of rays by this thickness of air probably does not introduce a significant error into these experiments.

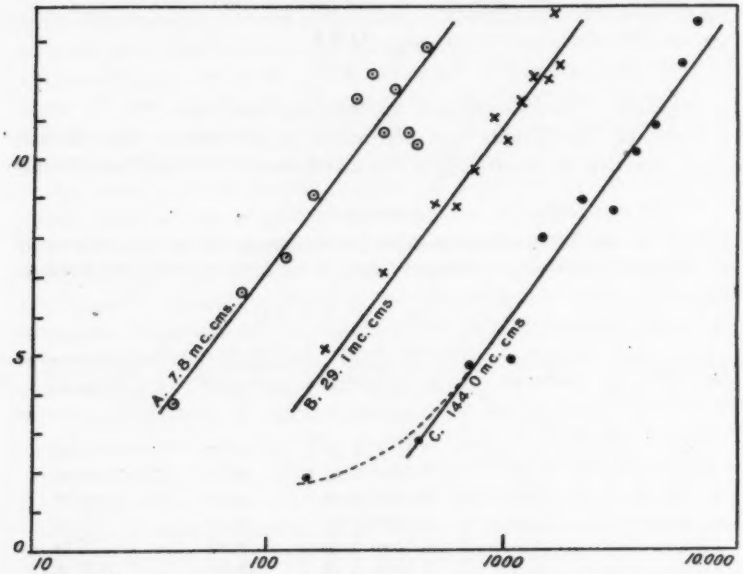

Fig. 2. Curves illustrating the influence of intensity of radiation upon the amount of swelling of the membranes of Nereis eggs produced by equal quantities of radiation. Quantities of radiation in millicurie centimeter minutes are measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

A. (September 13, 1917) represents the effect of radiating eggs from a single female for various periods of time with an intensity of 7.8 mc. cm.

B. (September 5, 1917) illustrates the data presented in table 4. Intensity 29.1 mc. cm.

C. (September 6, 1917) represents data from a similar experiment made with an intensity of 144 mc. cm.

Longer periods of radiation are however *relatively* less effective than shorter periods. The volume of the membrane varies directly with the logarithm of the time of radiation. The relationship may be expressed by the equation

$$V = A + c \log t$$

where V is the volume of the membrane resulting from radiation for a given time t, and A and c are constants depending on the experimental conditions, e.g., intensity of radiation, temperature, time elapsing between fertilization and measurement of membrane, etc. Values

for V calculated from these data, taking $A = -1.6$ and $c = 8.12$, are included in table 4. These values are in good agreement with the observed values. In figure 2, B is a curve based upon these data.

TABLE 5

Nereis eggs radiated for periods which varied inversely with the intensity employed
September 8, 1917. Measured sixty to seventy minutes after fertilization
$a = -0.02$ $b = 1.128$ $c = 3.75$

NUMBER OF EGGS MEASURED	INTENSITY	TIME	INTENSITY X TIME	VOLUME OF MEMBRANE OBSERVED $10^6\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^6\mu^3$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	millicurie centimeters	*minutes*	*millicurie centimeter minutes*			
25	8.94	64.8	578	8.40 ± 0.19	7.84	+0.56
25	24.4	34.3	837	6.60 ± 0.19	7.29	−0.69
25	26.7	23.4	625	5.98 ± 0.08	6.72	−0.74
25	37.3	15.0	560	6.65 ± 0.12	6.16	+0.49
25	71.5	7.9	565	5.13 ± 0.13	5.43	−0.30
25	172.0	3.4	585	5.17 ± 0.14	4.49	+0.68
September 10, 1917. Measured ninety to one hundred minutes after fertilization $a = 0.068$ $b = 0.67$ $c = 4.03$						
25	6.25	72.5	453	7.80 ± 0.25	8.10	−0.30
25	11.7	40.0	468	6.80 ± 0.09	7.24	−0.44
26	16.0	27.5	440	6.60 ± 0.26	6.57	+0.03
25	28.0	16.0	448	5.42 ± 0.16	5.34	+0.08
13	47.7	8.9	424	5.45 ± 0.43	5.01	+0.44
25	71.6	5.4	387	4.10 ± 0.16	4.25	−0.15
25	120.5	3.6	434	4.68 ± 0.20	3.73	+0.95
September 12, 1917. Measured ninety to one hundred minutes after fertilization $a = 0.017$ $b = 0.895$ $c = 3.674$						
25	12.4	55.0	682	7.84 ± 0.12	7.45	+0.39
25	23.1	29.4	682	6.06 ± 0.13	6.65	−0.59
16	36.2	19.3	698	6.62 ± 0.17	6.14	+0.48
25	54.9	12.4	681	6.05 ± 0.19	5.59	+0.46
25	95.8	7.05	675	3.62 ± 0.09	4.91	−0.29
25	162.5	4.20	683	5.27 ± 0.11	4.29	+0.98
23	251.0	2.72	683	3.22 ± 0.13	3.76	−0.54

One might expect the effect of radium radiations upon protoplasm to be a linear function of the product of intensity and time; that is, to be proportional to the number of rays striking the cell irrespective

of their distribution in time. That this relation is not true is clearly indicated by the subsequent data. In table 5 three experiments are recorded in which the time of radiation and its intensity were so varied as to keep the product of the two approximately constant. On the above supposition the volume of the membranes of the eggs in each experiment should have been the same. This result was not realized, the membranes being more voluminous with long exposures to low intensities than with short exposures to high intensities. The same condition is indicated by the data illustrated in figure 2. Here the volume of the membranes resulting from three experiments made with three different intensities of radiation are plotted against the logarithms of the quantity ($I.\ t$) of radiation. If the volume were a function of the product of intensity and time these curves should be superimposed. This is clearly not the case; the curve, A, made at the lower intensity indicates a much greater volume for a given quantity of radiation than do the curves, B and C, made at higher intensities.

A consideration of additional data has suggested that the equation relating intensity, I, and time, t, of radiation with the resulting volume, V, has the form

$$V = a + b \log I + c \log t$$

in which a, b and c are constants and b *is less than* c.

The best representative value for these constants can be determined for any given set of data. From the figures recorded in table 5 the membrane volume to be expected for each combination of intensity and time has been calculated. Although the deviation of the observed values from the calculated is considerable, in some cases exceeding 10 per cent, the deviations fall at random above and below the calculated values. Deviations of such magnitude are readily accounted for by errors in measuring the distance between the radium and the eggs. An error of 0.5 mm. in this measurement would cause an error of 5 per cent when the intensity was lowest and of 20 per cent when the intensity was highest.

A further test of the equation is afforded by measuring the volume of the membranes which result from exposing the eggs to various intensities of radiation for a uniform period of time. Such data are recorded in table 6 and in figure 3. Here a and $c \log t$ remain constant and V varies with $\log I$. The membrane volumes to be expected for the intensities employed have been calculated. Although this value agrees with the observed values in a quite satisfactory way the exact

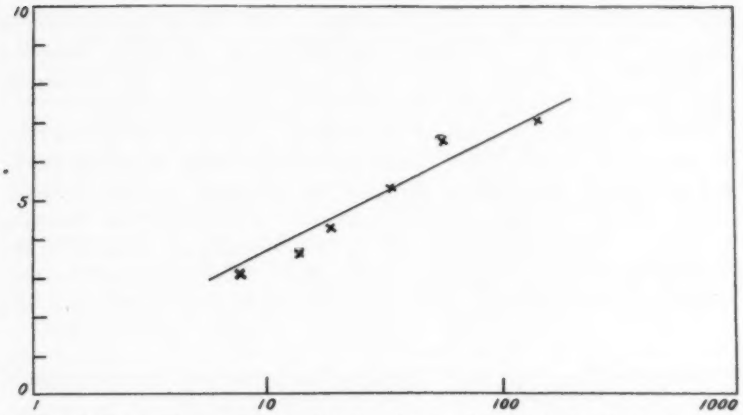

Fig. 3. Curve illustrating the data in table 6. Intensity of radiation in millicurie centimeters is measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

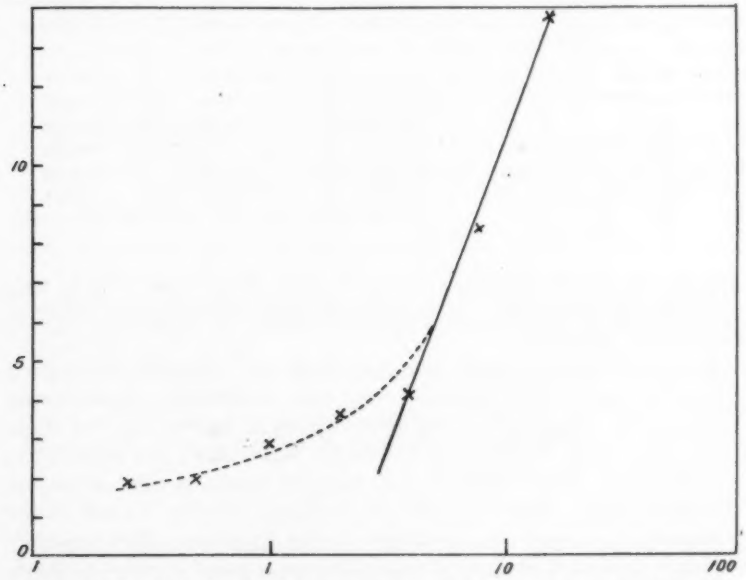

Fig. 4. The deviation in the lower range of the curve is indicated by data obtained by radiating Nereis eggs with 85 mc. cm. for short periods of time (September 12, 1917). Periods of radiation in minutes are measured logarithmically along the abscissa. Volumes of membranes are measured in 100,000 cubic micra along the ordinate.

form of the expression relating intensity to the rest of the equation is somewhat doubtful. Extensive data confirming the results recorded in table 6 are lacking, while errors in determining the intensities employed discount the value of such data which are at hand.

One fact stands out clearly from all data. *The value of b is less than the value of c.* This relation is indicated by the slope of the curves in figures 3 and 2 which are proportional to the value of b and c respectively, and by the values of these constants determined from the data in table 5. This relationship tells us that *quantity of radiation in the physical sense is physiologically meaningless.* The physiological action of radium radiations upon these cells does not depend simply upon the

TABLE 6

September 9, 1917. Nereis eggs radiated for fifteen minutes with varying intensity. Measured seventy to eighty minutes after fertilization

$a + c \log t = 0.68 \qquad b = 3.0$

NUMBER OF EGGS MEASURED	INTENSITY	VOLUME OF MEMBRANE OBSERVED $10^6\mu^3$	VOLUME OF MEMBRANE CALCULATED $10^6\mu^3$	VOLUME OBSERVED MINUS VOLUME CALCULATED
	millicurie centimeters			
25	7.54	3.15 ± 0.05	3.31	−0.16
25	13.78	3.65 ± 0.09	4.11	−0.46
25	18.70	4.30 ± 0.06	4.50	−0.20
25	33.70	5.35 ± 0.07	5.26	+0.09
25	57.52	6.55 ± 0.16	5.96	+0.59
25	145.2	7.05 ± 0.20	7.16	−0.11
13	0	1.80 ± 0.17		

number of rays or particles striking the cell; their distribution in time must also be considered. In this consideration time is a more effective factor than intensity.

In conclusion the range through which the equation holds good within the limits of experimental error may be defined. Experimental values of the volume of the egg above eleven or twelve hundred thousand cubic micra fluctuate considerably, but without any tendency in one direction. Below three or four hundred thousand cubic micra, on the other hand, there is a distinct tendency for the volume of the membrane to exceed that predicted by the equation. This tendency is clearly indicated in figure 4 and in the lower point in curve C, figure 2. In the lower range of the curve the values of a, b and c are no longer constant. Above this range the equation may be considered a

satisfactory approximation of the relationship of intensity and time to the swelling of the fertilization membrane of Nereis eggs.

The equation $V = a + b \log I + c \log t$ suggests that the change in the membrane is due to a process commencing after a latent period which is represented by the value of t when $V = 0$. The considerations of the preceding paragraph indicate, however, that the equation does not hold true at the beginning of the process. At this time the reaction evidently proceeds more slowly, as indicated by the slope of the curves, than the constants of the equation demand. A considerable time must elapse before the rate of change reaches a maximum. In this way a latent period which probably does not exist is suggested. The initial acceleration of the process finds a striking analogy in the phenomenon of photochemical induction which is said to occur in practically all photochemical reactions. Serious consideration of the nature of the processes in question should await, however, more complete demonstration of the relationship of the factors involved.[4]

[4] Striking, though perhaps superficial, resemblances exist between the action of light upon the photographic plate and the phenomenon here considered. Radiations from radium produce in the egg of Nereis a "latent image" which does not manifest itself until the egg is "developed" by fertilization. Moreover the change in the membrane is no more effected by the time elapsing between radiation and fertilization than the photographic negative is altered by the time elapsing between exposure and development.

Hurter and Driffield (4) have expressed the relation between the intensity, I, and time, t, of exposure of a photographic plate and the resulting density, D, of the negative—which is "directly proportional to the amount of silver deposited per unit area"—by the equation

$$D = \gamma \log \left(\frac{I\ t}{i}\right)$$

in which γ is a constant depending on the time of development and i is the "inertia" of the plate, "measuring those properties of the film which together constitute its sensitiveness."

If we let d represent the difference between b and c, then $c - d = b$ and we may rewrite our equation

$$V = a + (c - d) \log I + c \log t.$$

Rearranging we get a form of the equation,

$$V = a + c \log \left(\frac{I \cdot t^{\frac{1}{d}}}{I^{c}}\right)$$

which is strikingly like that of Hurter and Driffield. The density of the photographic plate after periods of *underexposure* also deviates from the expectation raised by their formula in a manner similar to the deviation of eggs radiated with small doses of radium.

Study of the subsequent history of radiated Nereis eggs indicates that a close parallelism exists between the abnormality in development and the change in the fertilization membrane. The data in table 7 show that similar quantitative relationships connect the degree of abnormality and the intensity and time of radiation. Although these lots of eggs have all received approximately the same quantity of radiation, those eggs which have been exposed for a long time to a low intensity have been much more affected than those exposed for a short time to a high intensity. This result suggests that a variety of cellular functions are affected in the same relative degree by intensity and time of radiation.

TABLE 7

September 12, 1917. Nereis eggs radiated for periods which varied inversely with the intensity employed. Membranes measured ninety to one hundred minutes after fertilization

INTENSITY	TIME	QUANTITY	VOLUME OF MEMBRANE $10^6\mu^3$	CONDITION OF LARVAE AFTER TWENTY TO TWENTY-FOUR HOURS
millicurie centimeters	*minutes*	*millicurie centimeter minutes*		
12.4	55.0	682	7.84	A few irregular cleavages. No swimmers
23.1	29.4	682	6.06	Irregular cleavage. No swimmers
36.2	19.3	698	6.62	Normal cleavage. No swimmers
54.9	12.4	681	6.05	ca. 5 per cent swimmers
95.8	7.05	675	3.62	ca. 50 per cent swimmers
162.5	4.20	683	5.27	ca. 50 per cent swimmers
251.0	2.72	683	3.22	ca. 90 per cent swimmers
0	0	0	1.8	ca. 90 per cent swimmers

The establishment of a mathematical relationship between quantity of radiation and amount of swelling should enable this reaction to be used as a measure of radioactivity. Heretofore our only methods of quantitating the intensity of radiation reaching a given point have been physical. Were the radiations homogeneous in nature such measurements would do very well as a basis for physiological investigation. Not only do the radiations vary qualitatively, but ratliations of any sort have various penetrating powers. There is no reason to suppose that a direct proportionality exists between the physiological effects of these different rays and their action upon the physical instruments used in measuring them. It should now be possible to

measure the intensity of those rays which alone are physiologically efficient, and thus to arrive at a rational basis for dosage in radiotherapy.

It is interesting to find, in the action of radium radiations the same logarithmic relationship as appears in the Weber-Fechner law relating stimulus and response. Davey (5) has found that a similar relationship determines the resistance of the beetle, Tribolium confusum, to death from various lengths of exposure to x-rays of a uniform intensity. Davey also observed a deviation from the expectation raised by the equation when the exposure was short. A similar deviation in the lower range of the curve is recorded by Henri et Larguier des Bancels (6) in experiments in which light was the stimulus.

SUMMARY

1. The fertilization membrane of the egg of *Nereis limbata* becomes abnormally thick if the egg has been exposed to radiations from radium prior to fertilization. This reaction is well adapted to quantitative study.

2. The change leading to this condition is irreversible.

3. The physiological effect is not proportional to the product of intensity and time. The time factor is relatively more important than the intensity factor.

4. An equation is suggested which expresses approximately the relation between intensity and time of radiation and their physiological effects.

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THE MECHANISM OF THE ACTION OF ANAESTHETICS

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Many theories have been advanced in attempts to explain how anaesthetics produce anaesthesia. Shortly after the discovery of ether and chloroform anaesthesia, Bibria and Harles (1847) called attention to the fact that practically all fat solvents are anaesthetics and as a result of a quantitative estimation of the fat contents of the brain of a normal animal and of a narcotized one, came to the conclusion that narcosis is produced by the direct removal of the fatlike substances, or lipoids, from the brain. The difficulty of explaining the rapid recovery which follows the interruption of anaesthetization and the fact that no one has been able to confirm the observations of Bibra and Harles, seems to have rendered their theory untenable. Hermann (1866) showed that all the narcotics of the methane series hemolyze the red blood corpuscles. He attributed this property to the power of these narcotics to dissolve the lecithin of the red blood cells, and assumed that narcosis of the central nervous system, with its high lecithin and cholesterin content, was due to the dissolving of these substances by the narcotics. Richet (1895) contended, on the contrary, that the effectiveness of a narcotic stood in an inverse relation to its solubility in the water fluids. The foregoing observations suggest the modern theory as set forth by Meyer and Overton. Hans Meyer (1) and Overton (2), independently, pointed out that the intensity of a narcotic is directly proportional to its distribution coefficient between the lipoids of the nervous system and the watery fluids; that is, the more soluble the narcotic is in the lipoids, the more effective it is as an anaesthetic. According to this theory, the narcotics of the methane series produce their characteristic effect on the central nervous system by going into solution in the fatlike constituents, the lipoids of the nervous tissue, and forming a physical-chemical combination with them. It should be mentioned, in this connection, that the Meyer-Overton law holds only for the narcotics of the methane series. The

dissolving of the lipoids of the nerve cells by these narcotics is supposed to alter the function of these cells, thus producing narcosis. It will be noted that while this theory explains very satisfactorily how a certain class of narcotics, namely those of the methane series, obtains access into the interior of the red blood corpuscles and of the nerve cells, it does not explain so satisfactorily the main point at issue, namely, how the narcotic produces narcosis. The fact that there are so many anaesthetics which are not fat solvents, magnesium sulphate and nitrous oxide being conspicuous examples, would seem to indicate that the Meyer-Overton theory, after all, may explain nothing more than how the narcotics of the methane series gain access into the nerve cells.

R. S. Lillie (3) has presented evidence showing that excitation is associated with increased permeability of the cell membrane, and depression with decreased permeability. According to this hypothesis, narcotics produce their characteristic effect by decreasing the permeability of the cell membrane. Claude Bernard (1853) suggested that narcosis was due to the semi-coagulation of the protoplasm, analogous to muscle rigor produced by chloroform.

It has been recognized for a long time that oxygen deprivation or asphyxia favors or may actually produce anaesthesia. So far as I have been able to find, John Snow, in his classical work, *On Chloroform and Other Anaesthetics* (1858), was the first to suggest that narcotics may produce narcosis by limiting or interfering with the normal oxidative processes. After reviewing the different theories of narcosis, Hewitt, in his book on *Anaesthetics* (1907), states that it is not at all improbable that future experimental research may lead us to the conclusion that general anaesthetics produce their characteristic effect by limiting the normal processes of oxidation, upon which the intellectual, sensory and motor centers depend for the execution of their respective functions. Paul Bert (4) and Arloing (5), independently, showed that oxidation was decreased during chloroform anaesthesia, as was indicated by the decreased oxygen intake and carbon dioxide output. Alexander and Cserna (6) showed that oxygen consumption and carbon dioxide production, and hence oxidation, in the brain was greatly increased during the excitement stage of anaesthesia, and was decreased during the stage of deep narcosis. Verworn (7) and his pupils have furnished much evidence showing that narcosis is usually accompanied by decreased oxidation, and that deficiency of oxygen, or asphyxia, as has been recognized for a long time, produces anaes-

thetic phenomena, hence they conclude that narcosis is due to the inhibition, or interference, with oxidation. Allied to this view is that of Mansfield (8) who assumes that narcotics decrease the solvent power of lipoids for oxygen, and hence prevent or interfere with its entrance into the cells. A. P. Matthews (9) believes that anaesthetics "fix the oxygen receptors of the protoplasm into a non-irritable anaesthetic-protoplasm combination." Herter found that the oxidizing capacity of the tissues was greatly reduced during anaesthesia. Tashiro found that anaesthetics greatly diminished the carbon dioxide output of nerves.

From this brief survey of the principal theories of narcosis, it would seem that the one of Verworn, which attributes narcosis to an interference or inhibition of the normal oxidative processes, is as plausible as any, if not the most plausible. In our work (10) we have shown that when oxidation was increased, as, for example, by increasing the amount of work, by thyroid feeding, by fighting, during the excitement stage of ether anaesthesia, there was an accompanying increase in catalase, due to the stimulation of the liver to an increased output of this enzyme, and that when oxidation was decreased or rendered defective, as, for example, by decreasing the amount of work, by starvation, by phosphorous poisoning, by extirpation of the pancreas, thus producing pancreatic diabetes with resulting defective oxidation, and in deep ether anaesthesia there was an accompanying decrease in the catalase of the tissues. From these results it was concluded that catalase, an enzyme in the tissues possessing the property of liberating oxygen from hydrogen peroxide, may be involved in the normal oxidative processes of the body. If it can be shown that the different narcotics decrease the catalase of the blood and hence of the tissues parallel with the decrease in oxidation during narcosis, it would seem to render it still more probable that catalase is involved in the oxidative processes and that the cause of the diminished oxidation, which is probably responsible for the narcosis, may be due to the decrease in catalase. The narcotics used were ether, chloroform, chloral hydrate, nitrous oxide and magnesium sulphate. These widely different kinds of narcotics were chosen intentionally. The animals used were cats, dogs and rabbits. The catalase of the blood was determined by adding 0.5 cc. of blood, taken from the external jugular vein, to hydrogen peroxide in a bottle at 22°C., and as the oxygen gas was liberated it was conducted to an inverted, graduated vessel, previously filled with water. After the oxygen gas thus collected in ten minutes had been

reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. In determining the catalase of the dogs' blood, 50 cc. of hydrogen peroxide were used, owing to the low catalase content of the dogs' blood, while 250 cc. of peroxide were used with the cats' and rabbits' blood. The material was shaken in a shaking machine at a fixed rate of one hundred eighty double shakes per minute during the determinations. The results of the determinations are given in figure 1. The figures (0–360) along the abscissa indicate time in minutes; the figures (0–900) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

Curve *1* was constructed from data obtained from four cats during ether anaesthesia. The anaesthesia was produced by bubbling air through ether in a bottle, which was connected by a rubber tube to a cone adjusted to the snout of the animal. It will be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the production of anaesthesia, was 875 cc. and 870 cc.; that after fifteen minutes of administration of the anaesthetic, 0.5 cc. of blood liberated 850 cc.; after thirty minutes, 835 cc.; after forty-five minutes, 790 cc.; after sixty minutes, 720 cc.; and after seventy-five minutes, 700 cc. From these figures it may be seen that the catalase of the blood was decreased parallel with the increase in the depth of narcosis, and that after seventy-five minutes it had been decreased by 20 per cent as indicated by the decrease in the amount of oxygen liberated from 875 cc. to 700 cc.

Curve *2* was constructed from data obtained from two cats during chloroform anaesthesia. The chloroform was administered in the same manner as was the ether. It may be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the anaesthesia, was 820 cc. and 820 cc.; that after fifteen minutes of administration of chloroform, 0.5 cc. of blood liberated 640 cc. By comparing the effects of ether and chloroform, it will be noted that there was a gradual decrease produced in the catalase of the blood during ether anaesthesia, whereas there was a very abrupt decrease during chloroform anaesthesia; that is, chloroform destroys the catalase of the blood more quickly than does ether. It was also found that with equal concentrations, chloroform destroyed the catalase of the blood more quickly and extensively in vitro than did ether.

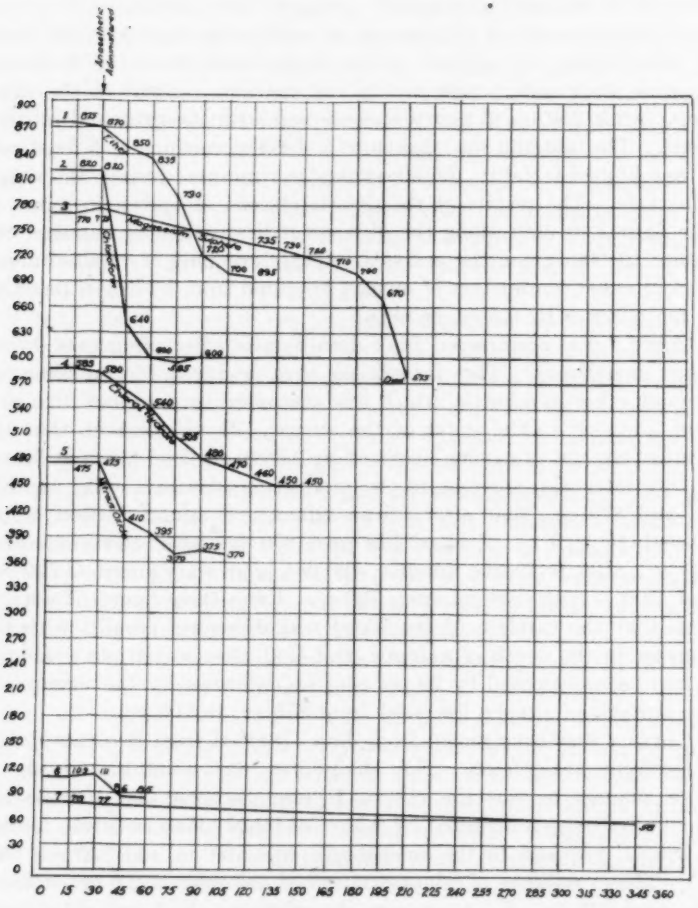

Fig. 1. Curves showing the effect of narcosis on the catalase content of the blood. The figures (0–360) along the abscissa indicate time in minutes; the figures (0–900) along the ordinate indicate amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

In the preceding experiments no attempt was made to administer the anaesthetic in equimolecular concentrations. Both were administered in sufficient concentrations to produce a fair degree of anaesthesia by the end of the first fifteen minute interval, and the amount administered during the remaining periods was such as to keep the animal in fairly deep but safe narcosis. We found that by choosing large, active cats with blood of high catalase content, and by forcing the anaesthetic, it was possible to decrease the catalase much more quickly and extensively than was done in the preceding experiments, but even in these cases it was found that chloroform produced a much more abrupt decrease than did ether, and the decrease, as a rule, was slightly greater with chloroform than with ether, particularly when the narcosis was continued over a period of two or three hours.

Curve *3* was constructed from data obtained from a cat during magnesium sulphate anaesthesia. The anaesthesia was produced by the subcutaneous injection of 7.5 cc. of a 20 per cent magnesium sulphate solution per kilo of body weight. It will be seen that the average amount of oxygen liberated by 0.5 cc. of blood taken at fifteen minute intervals, previous to the production of anaesthesia, was 770 cc. and 775 cc.; that ninety minutes after the injection of the magnesium sulphate, 0.5 cc. of blood liberated 735 cc. of oxygen; after one hundred thirty-five minutes, 730 cc.; after one hundred fifty minutes, 720 cc.; after one hundred sixty-five minutes, 710 cc.; after one hundred eighty minutes, 700 cc.; after one hundred ninety-five minutes 670 cc.; and after two hundred ten minutes, 575 cc.; when the animal died. It will be noted that the catalase was decreased more slowly during magnesium sulphate narcosis, except the abrupt decrease just preceding the death of the animal, than during narcosis produced by any of the other narcotics. It was also found that magnesium sulphate was the least effective of the narcotics used in destroying the catalase in vitro.

Curve *4* was constructed from data obtained from two rabbits, during chloral hydrate anaesthesia. The anaesthesia was produced by the introduction into the stomach of the animals of 10 cc. of a 2 per cent solution of chloral hydrate, per kilo of body weight. It will be noted that chloral hydrate decreased the catalase of the blood during narcosis more slowly than any of the other narcotics, except magnesium sulphate. It was found that when chloral hydrate was added to blood in vitro in as large quantities as was the magnesium sulphate, it de-

stroyed the catalase more quickly and extensively than did the magnesium sulphate, but less extensively than did the ether or chloroform.

Curve *5* was constructed from data obtained from two cats during nitrous oxide anaesthesia. The anaesthesia was produced by administering a mixture of nitrous oxide and oxygen in the proportion of one to five, or 80 per cent nitrous oxide and 20 per cent oxygen. It will be noted that the decrease in catalase was more abrupt with nitrous oxide than with any of the other anaesthetics, except chloroform.

Curve *6* was constructed from data obtained from a dog, during chloroform narcosis. The chloroform was administered in the same manner as it was with the cats for curve *2*. The same abrupt decrease in catalase during the first fifteen minutes of narcosis was obtained with the dog as was obtained with the cats.

Curve *7* was obtained from a dog, chloretonized in the same manner as were the rabbits for curve *4*. It will be noted that the same gradual decrease in catalase was obtained with the dog as was obtained with the rabbits.

If narcosis be due to decreased oxidation, and if this decreased oxidation, in turn, be due to a decrease in catalase, then the destructive effect of an anaesthetic on catalase should be an index to the character of the anaesthesia produced by the anaesthetic in question. It may be seen in the chart that chloroform, in keeping with its more powerful action as an anaesthetic, is more destructive to catalase than any of the anaesthetics used, and that magnesium sulphate, in keeping with its slow action, is least destructive, while ether occupies an intermediate position. Chloral hydrate does not act so rapidly as ether, chloroform or nitrous oxide, nor does it act so slowly as magnesium sulphate. It may be seen that chloral hydrate, accordingly, destroys catalase during narcosis less rapidly than ether, chloroform or nitrous oxide, and more rapidly than magnesium sulphate. It is known that a state of acidosis is more likely to develop with chloroform than with ether. It may be that the greater tendency toward acidosis in chloroform narcosis is due to the greater destruction of catalase by this narcotic, and to the injury of the liver, the organ in which catalase is formed, with the resulting decrease in oxidation.

SUMMARY

1. Narcotics of widely different constitution, such as chloroform, ether, chloral hydrate, nitrous oxide and magnesium sulphate, decrease the catalase of the blood, parallel with the increase in the depth of narcosis.

2. A very powerful anaesthetic, such as chloroform, decreases the catalase more quickly and extensively than does a less powerful anaesthetic, such as ether. Slowly acting anaesthetics, such as chloral hydrate and magnesium sulphate, decrease, accordingly, the catalase of the blood more slowly than a quickly acting anaesthetic such as nitrous oxide.

3. As a result of the experiments reported in this paper, and of work done previously on the anaesthetics in this laboratory, the theory is advanced that narcosis is due to the direct destruction of catalase by the narcotic, with resulting decrease in oxidation, while recovery from anaesthesia is brought about by an increase in catalase due to the increased output from the liver, with resulting increase in oxidation.

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THE RELATION BETWEEN GROWTH CAPACITY AND WEIGHT AT BIRTH

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The statistical data concerning the course of human growth during the first few days of extra-uterine life are voluminous and collectively significant. The individual reports however are unfortunately complicated by a certain degree of non-recognition of the fact that human milk is the proper food for human infants, and with the consequent inclusion in the data of growth curves of infants whose nourishment has been derived from other sources.

It is a matter of common knowledge (1) that the chemical composition of the milk produced by the various types of mammals is regularly different. It is also well known that, by and large, young mammals thrive better when feeding on the milk produced by their kind. No experimental evidence has as yet been presented in support of a theory that the milk elaborated by the mother is not that biologically adapted to the needs of the young of the same species. There apparently exists in mammals a mechanism for the production of a food specifically adapted to the needs of the young of the same kind. Moreover Osborne and Mendel (2), McCollum (3) and others have shown that the nature of the food ingested is one of the very important factors concerned in growth, and any attempt to determine the fundamental biological laws of growth during this period should recognize these facts.

The factors influencing milk production are manifold. The nutritive condition of the mother (4), the maternal metabolism, health or disease (5), all contribute their quota to the value of the milk as a food supply adequate to the growing infant. Nevertheless during the first two weeks of lactation the variations in the chemical composition of human milk have a remarkably uniform tendency (5) and where the changes in the weight of the infant do not indicate supplementary feeding necessary it can reasonably be assumed that the nourishment is sufficient and characteristic.

Growth is a bio-chemical process (6), (7), (8), (9), and as such is obviously susceptible to the influence of regulatory or interfering factors. Quantity of food ingested (10), climate, nationality (11), sex (12), and a variety of other conditions serve to produce a composite picture of such apparent intricacy that as late as 1913 Kjölseth (13) considered statistical studies on growth to be so hopeless as to propose the motto: "Die Natur is nicht schematisch." Fortunately the results of the studies of a long line of investigators extending from 1716 (14) to the present (15), (16) refute this unorderly point of view. A comprehensive bibliography of the work with infants up to 1913 is given by Benestad (11). No specific attempts were made to correlate birth weight and rate of growth, any such isolated observations as were reported yielding conflicting opinions; Schäffer (17) considering—"und so leichter ist das Kind . . . . um so länger dauert es, bis dasselbe sein Geburtsgewicht wieder erricht hat," and Benestad (11) that "die kleinen Kinder erleiden einen geringeren Gewichtsverlust und beginnen ihren Zuwachs eher als die grossen Kinder. Aber von dem Augenblick an, da die Gewichtzunahme einsetzt, besteht kein nennenswerter Unterschied zwischen ihnen."

Anticipating that a detailed study of the relation between weight at birth and early growth would bring out some significant and interesting differences, and having in mind as a fundamental requisite for normal growth a generically adapted nourishment, data were collected from the records of the Boston Lying-In Hospital of the weights of five hundred and thirty-seven infants on the 1st, 3d, 5th, 7th, 9th, 11th and 13th days after birth, excluding from consideration those whose food supply was derived either wholly or in part from sources other than the maternal breasts. Due care was taken throughout as to uniformity of conditions when weighing.

The classification is as follows:

GROUP	WEIGHT	NUMBER OF SUBJECTS
	pounds	
A	5–6	100
B	6–7	100
C	7–8	100
D	8–9	100
E	9–10	100
F	10–11	37

Infants under 5 pounds could not be included in the calculations inasmuch as they invariably received feedings supplementary to the mothers' milk.

Any comparative study of the power of growth in various groups of individuals is valid only when the changes occurring are considered from a percentage point of view. The absolute variations show the direction of the change but fail to give its value in terms of the original.

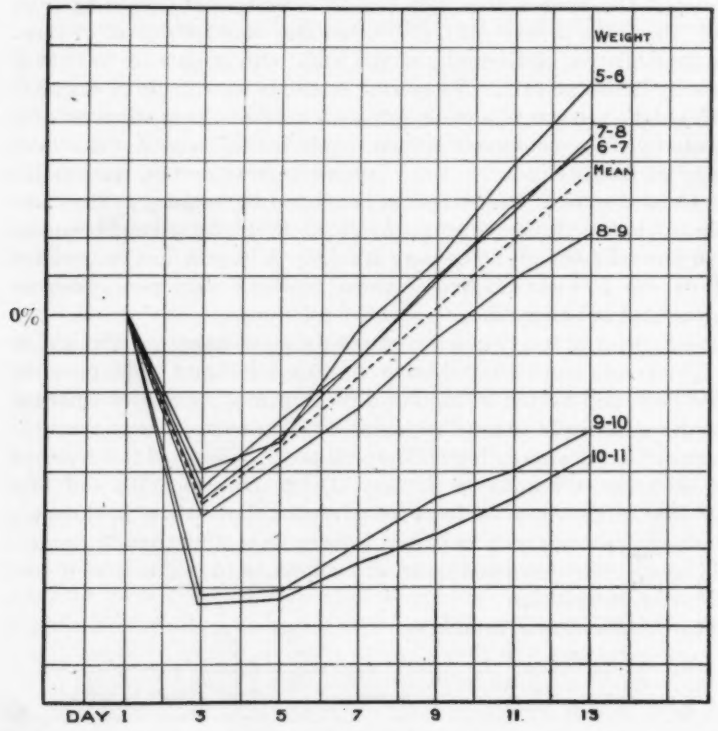

Fig. 1.

The results recorded in this paper are based on this fact and represent the per cent variations in the weights of the infants based on the weight at birth.

Table 1 gives the per cent change in body weight of the six groups of subjects during the period studied. These data have been plotted on figure 1.

There is at once made evident the division of the groups into two general classes, the members of which are governed by factors of similar intensity characteristic for the class. The groups A, B, C and D are seen to be closely alike throughout and distinct from the groups E and F. The causes of this differentiation are not at present explicable.

The post-natal decline mentioned by Quetelet (18) in a quotation from Chaussier, and the subject of much speculation, is here shown to vary in a remarkably uniform manner, according to the variation in the initial weight. The heavier the initial weight the greater the per cent drop in weight after birth. The post-natal per cent loss of weight varies directly with the weight at birth.

TABLE 1

The per cent change in weight from the first day during the first thirteen days after birth of the infants at the Boston Lying-in Hospital

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	−4.0	−3.3	−0.4	1.4	3.9	6.0
B	6–7	−4.7	−3.2	−1.1	0.9	2.8	4.4
C	7–8	−4.4	−2.7	−1.1	1.0	2.7	4.6
D	8–9	−5.1	−3.8	−2.4	−0.6	0.9	2.1
E	9–10	−7.2	−7.1	−5.9	−4.7	−4.0	−2.9
F	10–11	−7.4	−7.3	−6.4	−5.6	−4.7	−3.6

This decrease which occurs during the first two or three days after birth is the physiological response to the radical change in the methods of food assimilation and ingestion. During this period of readjustment the catabolic processes are superior to the anabolic with the resultant utilization of body tissue for maintenance. Pending the efficient establishment of a functional activity these destructive reactions overbalance the effect of the growth stimuli, if such are present at this stage, and no increase in weight occurs.

The growth catalyzers are apparently lipoidal in nature (19) and one of them has been recently isolated and named "Tethelin" (20), (21). Similarly acting substances have been found in various foods (22). It is possible to consider that those animals a part of whose development occurs in the uterus receive the necessary stimuli to growth from the maternal blood or placental secretions (23), and ample evidence has been presented that an organ may be developed to a point where

it is capable of assuming its normal function yet does not do so, or does so only at a minimum rate, until the call for functional activity is thrust upon it. Now at birth the infant is cut off from the maternal exogenous stimulation to growth, and simultaneously is readjusting itself to changed methods of nutrition. Pending this readjustment the ability to make visible growth will depend on the relative intereffect of stimulus and catabolic processes. Those organisms in which there is proportionately less substrate for activation, from whatever source, would tend to have a lesser loss of weight. Minot (12) has shown that during intra-uterine life there is an enormous loss of growth capacity. It appears to be a fact however that the first growth cycle is not quite completed at birth and that an increased growth rate occurs at or near birth (6), therefore the most effective stimulation to growth would

TABLE 2

The per cent change in weight from day to day during the first thirteen days after birth of the infants at the Boston Lying-in Hospital

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	−4.0	0.7	2.9	1.8	2.5	2.1
B	6–7	−4.7	1.5	2.1	2.0	1.9	1.6
C	7–8	−4.4	1.7	1.6	2.1	1.7	1.9
D	8–9	−5.1	1.3	1.4	1.8	1.5	1.2
E	9–10	−7.2	0.1	1.2	1.2	0.7	1.1
F	10–11	−7.4	0.1	0.9	0.8	0.9	0.9

occur in those lighter individuals who have failed to reach the normal point in intra-uterine development, and the counterbalancing effect of the catabolic processes would be diminished in an effort to respond to the greater stimulus.

With the exception of groups E and F the third day after birth marks the beginning of the pick-up to the normal rate of growth characteristic for the individual groups. The heavier infants do not begin this increase either as soon or to the same degree as do the lighter ones, this retardation effect is explained by an extension of the principles embodied in the previous discussion. From this time on the growth acceleration is practically uniform in value for any single group but diminishes with the increase in initial weight. It is significant of an underlying causative factor of growth inversely varying in intensity

of effect with the weight at birth that the various groups tend to individually attain a uniform percentage increment.

The differences from day to day of the per cent change in weight from the first day during the period of observation are given in table 2. They show the per cent additions made between the successive weighings.

The post-natal lag, extending to the fifth day, before the pick-up to the relatively uniform increment characteristic for the single groups, exhibited in groups A, E and F, at the extreme upper and lower limits of the weight at birth are indicative of a factor or factors retarding the early attainment of a normal rate of growth in these individuals. This phenomenon is not found in groups B, C or D, where although variations in the differences in the per cent increments do occur from day to

TABLE 3

The per cent recovery to or over the initial weight of the groups studied

GROUP	WEIGHT	DAY					
		3	5	7	9	11	13
	pounds						
A	5–6	19	29	50	62	75	82
B	6–7	8	24	45	60	75	80
C	7–8	12	24	39	60	74	78
D	8–7	7	17	30	49	60	70
E	9–10	2	5	15	20	30	35
F	10–11	3	3	5	8	11	20

day, yet they are relatively negligible even from the onset of demonstrable growth, and especially when compared with the marked retardation effect shown in the other groups. It is possible that a condition of instability between catalyst and substrate due to the radical change in environment of the individual as a whole is the cause of this condition in group A, and this seems more probable when we look at the subsequent variations in this value during the remainder of the period. The delay in picking up to the rate of growth normal for the group in the heavier infants is the expression of either a diminished response to the growth stimulus due to the increase in substrate, or to the preponderant effect of the catabolic processes over those initiated by the growth catalysts. The former hypothesis is the more attractive.

Continuing the inspection of this table it is seen that the variations in the per cent increment from day to day grow smaller as the weight at

birth increases. This diminution in absolute per cent increment is not sufficiently compensated for by the differences in initial weight to cause an equivalent growth acceleration in all groups. It can be stated with certainty that the heavier the initial weight the slower the rate of growth.

Camerer's (24) results seem to indicate that these differences in growth are similarly correlated with the differences in initial weight even throughout a much longer period for he found that in a series of one hundred and thirty-eight cases divided into three groups, according as they weighed under 2000 grams, between 2000 and 2750 and over 2750, and studied after weekly weighings, that the percentage increment of the subjects in the first group was 427, of the second 219 and of the third and heaviest only 195.

TABLE 4

The per cent distribution according to the weight at birth of one thousand consecutive cases at the Boston Lying-in Hospital

GROUP	WEIGHT	PER CENT
	pounds	
A	5–6	8.6
B	6–7	30.3
C	7–8	34.7
D	8–9	19.8
E	9–10	5.8
F	10–11	0.8

The extension of these facts as given by the data calculated for this paper clearly indicates that the mass or weight of the infant at birth is a determining factor in the subsequent rate of growth.

As a corollary to the foregoing, table 3 shows that the per cent of the subjects recovering or passing their initial weight after the postnatal decline is regularly influenced by the weight at birth, the retardation effect of an increased initial weight is here particularly well demonstrated.

The mean increment per cent for the six groups has been calculated and plotted on figure 1. The close approximation of this value to a straight line lends support to Osborne's (25) citation as to the applicability of Newton's first law to biological phenomena.

To obtain the figures necessary in calculating the mean, record was made of the weight of one thousand consecutive subjects and classified accordingly. Table 4 gives the per cent distribution in the six groups.

A correlation of the fact that 65 per cent of all infants weigh between six and eight pounds at birth, with the coincidental character of the growth curves of these two groups as shown on the chart, together with the fact that the curve of the mean is practically parallel with these curves leads to the idea that the normal birth weight lies between these limits and that the normal growth acceleration follows the indicated direction.

If increments of matter are used as a measure of growth it is obvious that in order to obtain a fair idea of the relative growth capacity of the various groups it is necessary to use that weight as a basis for calculation to which demonstrable additions are being made. With this point in mind there has been calculated the per cent increment in weight from the third day after birth. This is given in table 5. The third

TABLE 5

The individual growth capacity and the relative growth capacity of human infants classified according to their weight at birth

GROUP	WEIGHT	INCREMENT FROM THIRD DAY	CAPACITY	RELATIVE CAPACITY
	pounds	*per cent*	*per cent*	
A	5–6	10.0	1.818	100
B	6–7	9.1	1.400	77
C	7–8	9.0	1.200	66
D	8–9	7.2	0.847	47
E	9–10	4.3	0.453	25
F	10–11	3.6	0.343	19

column shows the per cent addition capacity of one pound of body weight at birth for the first thirteen days of extra-uterine life. The last column shows the relative growth capacity of the groups studied when group A is used as unity.

Having thus brought growth capacity to a unit basis and with the idea that the per cent increment that can be made by unit weight is an index of the capacity to grow, we find that the ability to add to the initial weight decreases with the increase in initial weight. That is to say, one pound of body weight of an infant weighing from eight to nine pounds at birth can add on a greater proportion of itself than can one pound of an infant weighing from nine to ten pounds at birth, and less than can one pound of an infant weighing from seven to eight pounds initial weight.

What is the significance of this regular variation in growth capacity? If we agree with Minot (26) that the "more rapid growth depends on

the youth of the individual" we must conclude that the weight of an infant at birth is an index of its relative physiological age. Extending this to the data presented here would give rise to the opinion that a birth weight lying between six and eight pounds is indicative of the completion of the intra-uterine growth cycle, that weights under six pounds represent physiologically younger individuals, while those over eight pounds at birth have completed and passed this cycle and are physiologically older. It is a fact that in the several groups the variability is inversely roughly proportional to the weight at birth; a correlation of this with the fact that there occurs a diminution of variability as time goes on or with senescence (12) produces additional support for the above idea.

Now growth in large measure is dependent upon the mutual intereffect of the growth stimulus, food supply and the catabolic processes of metabolism. This has been expressed in part by Friedenthal (27) who states that "ein Lebewesen wächst solange die Zunahme der Masse der lebendigen Substanz in seinem Körper den Verbrauch an lebendigen Substanz durch die Lebenschädigungen im ganzen überwiegt." It is permissible to omit from discussion the reciprocal interdependency of metabolism and growth. The diets were substantially the same for all the mothers and the metabolic processes of the infants can be considered as sufficiently uniform in nature to require no further comment. This leaves the relation of mass to catalyst as the fundamental determinant of the growth capacity of the infants studied. Now Hatai's (28) expression of the idea that "an organism tends during growth to form the greatest amount of mass with the least loss of growth capacity" does not quite coincide with these results; in fact they seem rather to lend support to Robertson's conception of growth as an autocatalyzed reaction: for it is plainly evident that growth capacity and rate of growth are increased by a diminution in the total mass at birth and decreased by an increase of the total mass at birth; this is so not only from the relative point of view but also from the standpoint of absolute increments. Enriques' (29) opinion that "das Wachstum des Stoffes wird zur einschränkenden Ursach des Wachstums selbst" would also seem to be borne out by these figures and the retardation effect on the rate of growth of the increase in weight at birth is obviously the result of the preponderance of substrate over catalyst.

CONCLUSIONS

The growth capacity of human infants during the first two weeks after birth is in a large degree dependent upon the weight at birth. It

is roughly inversely proportional to the initial weight. The ability to recover and pass the initial weight after the post-natal decline obviously varies in the same way, so that at the completion of the period studied some 82 per cent of those infants weighing between 5 and 6 pounds at birth have recovered or passed their initial weight, as compared with 20 per cent of those weighing from 10 to 11 pounds. The intermediate groups vary inversely as to their weight at birth.

Thanks are due to the staff of the Boston Lying-in Hospital and to the office force and nurses for their unfailing courtesy and assistance in making possible the collection of the data herein discussed. The kindly criticisms of Dr. John L. Bremer have done much to make this material presentable.

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THE INCREASE OF PERMEABILITY TO WATER IN FERTILIZED SEA-URCHIN EGGS AND THE INFLUENCE OF CYANIDE AND ANAESTHETICS UPON THIS CHANGE

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I. INTRODUCTORY

The problem of the nature and conditions of cell-permeability is by no means a special or limited one but involves the whole question of the essential physico-chemical constitution of living matter. The plasma-membrane, or semi-permeable surface-layer of the cell, is not to be regarded merely as a simple passive partition separating the living substance from the surrounding medium, but rather as an integral part of the living protoplasm itself, characteristically modified in its physical properties and in its chemical composition and activities by the conditions at the cell-boundary. Some of its general peculiarities, such as the existence of a surface-tension, its greater viscosity or structural density as compared with the internal protoplasm and its difference in composition from the latter, are general properties of any surface-layer at the boundary between two immiscible fluids containing surface-active substances (especially colloids) in solution; hence the protoplasmic surface-layer has frequently been compared to a haptogen membrane. But no merely static conception of the structure and composition of this region of the cell is sufficient to explain all of its observed properties. Thus there is no doubt that its most general characteristic of semi-permeability—the all-essential insulating and diffusion-preventing property—is not merely the result of a special chemical composition and structural density, such as determine the semi-permeability of a precipitation-membrane, but is inseparable from the living condition, i.e., is actively maintained by a continual process of metabolism. The proof of this is that death—the cessation of metabolism—however caused, is invariably followed by a loss of semi-permeability, i.e., the normal state of the membrane then ceases to be maintained and the unhindered processes of diffusion lead to the disintegration of the

cell. Hence destruction of the surface-layer by artificial means—cytolytic substances, heat, extensive mechanical injury—is quickly fatal to all cells.

Since the normal properties of this layer are thus preserved by cell-metabolism, and are lost when metabolism ceases, it is not surprising to find that these properties vary with the state of metabolism, i.e., with the physiological activity of the cell. The impermeability which the plasma-membranes of most cells usually exhibit toward substances like sugar and neutral salts may thus temporarily disappear under certain conditions, and there is much evidence that this takes place especially at times of stimulation or increased functional activity;[1] the necessary entrance and exit of materials to which the membrane is at other times impermeable is thus rendered possible. Simple diffusion, however, is not sufficient to account for this interchange. We know that in the transport of substances into and out of cells in the processes of absorption and secretion osmotic work is performed, the energy of which is evidently derived from the chemical decomposition of cell-constituents; and in the normal entrance of food-substances and the exit of excretory materials in all cells similar factors are probably at work. Apparently it is by means of this physiological mechanism of transport, acting in association with temporary increase of permeability, that the normal interchange of materials with the surroundings is effected.

It is to be noted that this condition implies *intermittency* in the process of interchange, with corresponding intermittent variations in the osmotic properties of the plasma-membrane. The constant association of these variations of permeability with bioelectric variations, i.e., with electric currents passing between the cell-interior and the surroundings, suggests that processes of electrical convection or electro-endosmose are concerned in the transport. Bioelectric currents always accompany stimulation and functional activity, and must, like other electric currents passing through solid partitions, cause transport of fluid.[2] That the physiological transporting mechanism acts inter-

[1] *Cf.* the instances of secretion, activation of egg-cell, many stimulation processes. For a summary of the general evidence *cf.* my papers in this Journal, 1909, xiv, 24; 1911, xxviii, 197; 1915, xxxvii, 348.

[2] *Cf.* my recent paper in Biol. Bull., 1917, xxxiii, 135; see pp. 170 *seq.* In any bioelectric current the positive stream flows in the extracellular part of the circuit from the inactive to the active region; the flow of water in electro-endosmose is with the positive stream when the solid material composing the partition is negatively charged, as in living cells; hence the current will tend to convey water into the cell at the active region, which is also the region of increased permeability.

mittently seems certain from the above considerations; and this intermittency may at times become regularly rhythmical in character; the case of heart-muscle and other rhythmically acting tissues probably exemplifies this condition. It is possible that the wide distribution of rhythmical activities like ciliary movement is an index of a general tendency on the part of protoplasmic surface-structures to vary rhythmically in their permeability and electrical polarization.[3]

The theory that variations of permeability are constantly associated with functional activity explains the apparent paradox that the plasma-membranes of resting cells, e.g., muscle-cells, usually exhibit themselves in osmotic experiments as impermeable to just those substances which are most necessary for their continued life, viz., sugars, amino-acids and neutral salts; as we have just seen, such substances are probably transported across the cell-boundary by a physiological process acting intermittently, which is largely independent of diffusion. On this view, variations in the permeability of the plasma-membrane form an essential condition of interchange with the surroundings; and since such interchange is obviously necessary to continued metabolism, we reach the general conclusion that the control of metabolic processes depends largely upon these variations of permeability. The associated bioelectric currents may by means of their electrolytic action directly determine the chemical changes taking place at the cell-surface.[4]

On the other hand, the normal properties of the plasma-membrane itself, as of other cell-structures, are maintained by processes of constructive metabolism; these automatically replace the material which is altered or destroyed in activity or otherwise. The materials necessary for the reconstitution of the membrane-substance after breakdown are continually being synthesized and laid down as part of the organized structure; in this manner the properties of the membrane are kept constant and the stability of the living system is ensured. Of all cell-structures the plasma-membrane thus appears to be the most stable and resistant; for example, in the decrease of size incident to starvation it remains intact with unaltered properties, a fact showing that it must then maintain itself at the expense of the internal protoplasm. This fact again illustrates the special importance of the surface-film in the maintenance of the living system. There are various other indications that the *surface-metabolism* of living protoplasm is the controlling metabolism; it seems indeed probable that the prevalence of the

[3] Biol. Bull., *loc. cit.*, 169.
[4] Biol. Bull., *loc. cit.*, 172.

cellular type of structure in organisms, with the large development of surface-protoplasm which it makes possible, is ultimately to be referred to the existence of a general condition of this kind.[5]

The normal semi-permeability of the plasma-membrane thus appears to be maintained by a specific metabolic regulatory process, the precise nature of which is still far from clear but which automatically restores the semi-permeability of any region of the cell-surface whenever the latter becomes permeable to any of the essential water-soluble cell-constituents—i.e., whenever the continuity of the surface-film is interrupted. Hence this continuity tends to be regained quickly after any change involving alteration of the cell-surface.[6] On any other assumption it seems impossible to account for the characteristic insolubility of living protoplasm in the aqueous medium usually surrounding it; although typically in a fine state of subdivision (i.e., into numerous minute "cells"), protoplasm resists perfectly the solvent or disintegrative action of water, notwithstanding the high water-solubility of many of its constituents. This water-insolubility—which is shown by the existence of a sharply defined and permanent surface of separation between protoplasm and medium—constitutes, from the physico-chemical point of view, one of the most remarkable of its peculiarities. In order to account for this property it seems necessary to assume that the cell-surface consists chiefly of water-insoluble materials and also that materials of this nature are continually being formed in metabolism and deposited at the surface to replace those normally lost. The significance of the lipoid constituents of protoplasm becomes clearer on such a view; these substances have the solubilities of fats and are water-insoluble in the true sense, although readily forming colloidal suspensions or emulsions; hence they are probably chiefly responsible for the water-insoluble character of the surface-film. We may thus understand why the plasma-membrane is so effective a barrier to those water-soluble compounds (like sugar and neutral salts) which are also lipoid-insoluble, while readily admitting lipoid-soluble compounds—a general property of living cells the importance of which was first recognized by Overton.

One might suppose that a layer composed largely of water-insoluble material would also form a barrier to the passage of water, yet the general impression is that water enters and leaves living cells with

[5] Biol. Bull., *loc. cit.*, 184.

[6] *Cf.* the observations of Chambers showing rapid reconstruction of the surface-film in sea-urchin eggs after injury: this Journal, 1917, xliii, 1; *cf.* pp. 6 *seq.*

great ease. This however may be due to the large ratio of surface to volume in such minute structures as cells, rather than to a high specific permeability to water. It is noteworthy that living cells retain water with greater tenacity than dead cells, as shown by their more gradual loss of weight when exposed to evaporation;[7] and this fact indicates that the permeability to water undergoes a decided increase after death, coincidently with the general increase of permeability to dissolve substances. Bernstein's explanation is that in the living cell the electrically polarized condition of the plasma-membrane makes the outward passage of water difficult; but Höber opposes this view and suggests that the slower evaporation from living tissues is due simply to the presence of turgor; when the cells die and lose semi-permeability turgor also disappears, and water then for the first time leaves the cells readily and evaporates. There is however little if any turgor in the vertebrate tissues used in many of Bernstein's experiments; yet in these, as well as in plant tissues, the rate of evaporation is much increased by death. Why such evaporation should take place slowly through the living and rapidly through the dead plasma-membrane seems not easily to be explained except on the view that the living membrane offers a greater resistance to the passage of water, i.e., is relatively impermeable to water. In general a high degree of semi-permeability in artificial precipitation-membranes appears to require a high degree of impermeability to water, as Morse found in his determination of the osmotic pressure of sugar solutions.[8] That the almost perfect semi-permeability exhibited by many living plasma-membranes is in reality often associated with a correspondingly high impermeability to water may readily be shown in certain cases. For example, the rate of abstraction of water from unfertilized sea-urchin eggs in strongly hypertonic sea water is surprisingly slow, as I shall describe later; and the same is true of the rate of swelling in dilute sea-water, a fact to which both Harvey and I have recently called attention.[9] It is possible that the degree of permeability of the plasma-membrane to water may be a general index of its permeability to all substances which enter and leave the cell in aqueous solution, especially if this transport is normally

[7] *Cf.* Bernstein: Elektrobiologie, Braunschweig, 1912, pp. 165 *seq.*

[8] Morse: Osmotic pressure of aqueous solutions. Carnegie Institution, Washington, 1914. See Morse's remarks on the necessity of a fine texture in the porcelaine cells supporting the precipitation membrane, p. 15; also pp. 87 *seq.*

[9] Harvey: Science, N. S., 1910, xxxii, 565; R. Lillie: This Journal, 1916, xl, 249.

accompanied by a flow of water (as is the case, e.g., in secretory processes). Evidently there can be no interchange of dissolved material between two adjacent solutions separated by a solid partition if the solvent cannot pass the partition (unless indeed the partition itself also acts as a solvent); for example, a glass bottle containing a solution shows no osmotic effects when immersed in water. In general, the rate of any osmotic process is limited by the permeability of the membrane to the solvent[10]; and in view of the importance of osmotic processes in physiology, it seems desirable that the conditions of permeability to water, as well as to dissolved substances, should receive further investigation. Hitherto little attention has been paid to this general problem; in many cells, however, the degree of permeability to water is a constant and definite character, which varies with physiological conditions and can be measured with considerable accuracy.

In certain cases a quantitative expression of the permeability of the plasma-membrane to water may be obtained by measuring the rate at which water enters or leaves the cell under a definite gradient of osmotic pressure. This is done by determining the alteration in weight or volume taking place in a given time in a hypertonic or hypotonic physiologically balanced medium of known osmotic pressure. The cases where such a method can be expected to give reasonably accurate results are perhaps not numerous. To determine at frequent intervals the weight of a tissue immersed in an anisotonic medium is a difficult and often impracticable process with many sources of accidental variation. Consistent results are possible, however, in the case of spherical cells like sea-urchin eggs, which swell slowly in hypotonic media (e.g., dilute sea-water) without change of form. In such eggs the diameter at any time can be measured rapidly by the ocular micrometer with a sufficient degree of accuracy, and the volume can be calculated on the assumption that the form is spherical. Using this method, I was able to show that in the Arbacia egg fertilization is followed by an approximately fourfold increase in the permeability to water.[11] By this means it would probably be possible to compare the relative permeability of different species of eggs to water and to study the variations of permeability in the same egg under different conditions of temperature, physiological activity, composition of medium, etc. The ratio between the rate of entrance of water under standard conditions (of osmotic pressure-gradient, temperature, composition of

[10] *Cf.* Antropoff: Zeitschr. physik. Chem., 1911, lxxvi, 721.

[11] This Journal, 1916, xl, 249.

medium) and the area of the membrane would give a measure of the specific permeability of the membrane to water. In cases where this method proved applicable its simplicity would be an advantage.

II. DIRECT EFFECTS OF HYPERTONIC SEA-WATER UPON FERTILIZED AND UNFERTILIZED ARBACIA EGGS

In any hypertonic medium which is otherwise non-injurious (*i.e.*, free from toxic substances and containing the necessary salts in balanced proportions, like sea-water or van't Hoff's solution) Arbacia eggs show the usual behavior of living cells, they lose water and shrink; in a hypotonic medium they swell. In general the rate of the osmotic entrance or exit of water in any cell, after transfer from its normal medium to one of similar constitution but different osmotic pressure, varies directly (1) with the gradient of osmotic pressure between the interior and the exterior of the cell, (2) with the area of the enclosing semi-permeable membrane, and (3) with the permeability of this membrane to water. Hence if the same cell exhibits at different times definite inequalities in the rate of osmotic gain or loss of water in the same medium, the inference is that the resistance to the passage of water across the membrane has varied correspondingly,—in other words that the permeability to water is subject to change under varying physiological conditions. The Arbacia egg presents a very clear case of this kind, fertilization being followed regularly by a marked increase in the permeability of the plasma-membrane to water,—as may readily be shown by bringing the eggs into either dilute or concentrated sea-water; in the former medium they swell, in the latter they shrink, but in both cases the rate of the process is much greater in the fertilized than in the unfertilized eggs. Both swelling and shrinkage are surprisingly slow in unfertilized eggs; when these eggs are transferred from sea-water into a strongly hypotonic or hypertonic medium they exhibit little alteration of size at a time (e.g., one or two minutes after transfer) when fertilized eggs in the same solution are conspicuously swollen or shrunken (see fig. 1). This difference of behavior relates entirely to the *rate* at which water either enters or leaves the egg; the *degree* of swelling or shrinkage when osmotic equilibrium is reached does not differ appreciably in the two kinds of eggs.[12] It is clear therefore that the change in osmotic properties has nothing to do with any change which

[12] See the curves in my former article, *loc. cit.*, 255.

fertilization might be supposed to produce in the osmotic pressure of the egg-protoplasm, but is determined solely by the greater readiness with which water enters or leaves the fertilized egg.

According to my former measurements on the rate of swelling of fertilized and unfertilized eggs in dilute sea-water, the resistance to the passage of water through the plasma-membrane is decreased, as a result of fertilization, to approximately one-fourth of its former value. A second method of detecting and estimating the change in the permeability of the egg to water is to determine the relative rates

TABLE 1

CONCENTRATION OF SOLUTION	IMMEDIATE EFFECT OF SOLUTION	EFFECT OF RETURN TO SEA-WATER
1. 2.0m	Rapid and complete collapse with immediate and marked loss of pigment from the eggs	Eggs remain collapsed and do not cleave or develop
2. 1.5m	Collapse and loss of pigment are rapid, but less so than in solution 1	Most eggs remain collapsed, but a few (*ca.* 5 per cent) recover the normal water-content and cleave
3. 1.25m	Rapid shrinkage and crenation with some extraction of pigment, but less than in solution 2	Most eggs round off within 3 minutes and later cleave; the majority form blastulæ.
4. 1.0m	Eggs shrink and crenate more slowly than in solution 3; no evident loss of pigment in 2 minutes	All eggs round off rapidly and later continue cleavage and development; the great majority form larvæ
5. 0.75m	Shrinkage is slower than in solution 4 and relatively slight; all eggs are slightly crenated in one minute	All eggs form larvæ

of shrinkage in strongly hypertonic sea-water or van't Hoff's solution, and some of the possibilities of this method were investigated at Woods Hole last summer. In experiments of this kind it is important to avoid too great a degree of hypertonicity in the solutions used, since then injury results; on the other hand, if the osmotic pressure is too low the effects are not definite enough. The following experiments (table 1) will illustrate the nature of the effects observed with media of varying osmotic pressure. Fertilized eggs were placed, thirty to forty-five minutes after fertilization, in van't Hoff's artificial sea-water of the concentrations given in the table. The action of each solution upon

the eggs was observed in watch-glasses. After remaining in the solutions for ten to fifteen minutes the eggs were returned to normal sea-water, and the changes immediately following this second transfer were also observed. Some of the eggs were left in sea-water in order to observe later the effect of the treatment upon cleavage and development.

These experiments show that irreversible effects appear only when the excess of osmotic pressure approaches the order of twenty atmospheres (solution 2). The osmotic collapse in the first two solutions is permanent and is associated with a cytolytic action indicated by loss of pigment; the failure of the eggs to round off on return to sea-water shows that the semi-permeability of the plasma-membrane has been permanently destroyed; evidently some irreversible structural alteration has taken place. In the less concentrated solutions the osmotic properties of the membrane remain unimpaired, and the eggs recover their normal water-content in sea-water and continue development. Solutions having an osmotic pressure similar to that of solutions 2 and 3 were used in most of the following experiments. In most cases these solutions were made by mixing concentrated van't Hoff's solution with sea-water.

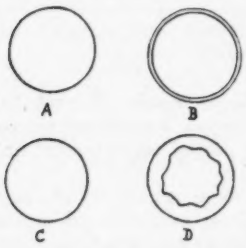

Fig. 1. *A* and *B*, outlines of unfertilized and fertilized Arbacia eggs in normal sea-water; *C* and *D*, appearance of the same eggs after one minute in hypertonic sea-water.

Differences between fertilized and unfertilized eggs. When transferred to hypertonic sea-water or van't Hoff's solution of 35 to 40 atmospheres osmotic pressure, fertilized eggs are at once seen to shrink rapidly and the egg-surface is thrown into characteristic folds and crenations; unfertilized eggs in the same medium shrink slowly and at first imperceptibly, and remain round. Any balanced medium of sufficient hypertonicity may be used to demonstrate this difference; in most of the following experiments a mixture of one volume of 2.5m van't Hoff's solution[13] *plus* three or four volumes normal sea-water was used; the former solution has an estimated osmotic pressure at 20° of *ca.* 40 atmospheres, the latter of *ca.* 36 atmospheres—as against the 24 atmospheres of the sea-water at Woods Hole. In either of these solu-

[13] Van't Hoff's solution contains the following salts in the molecular proportions: 100 NaCl, 2.2 KCl, 7.8 $MgCl_2$, 3.8 $MgSO_4$, 2 $CaCl_2$. The above solution is made by mixing 2.5m. solutions of the salts in the above proportions by volume.

tions fertilized eggs begin to crenate within fifteen or twenty seconds after transfer while unfertilized eggs show no evident change until much later. The most striking and convenient method of showing this difference is to place a mixture of equal numbers of unfertilized and fertilized eggs (the latter fertilized at least fifteen minutes previously) in hypertonic sea-water. The fertilized eggs at once shrink rapidly and undergo crenation, and within less than one minute exhibit a collapsed, shrunken and angular appearance; at this time the unfertilized eggs are apparently unaltered, so that a striking contrast is presented (see fig. 1). Shrinkage continues slowly in the unfertilized eggs and becomes well marked in the course of five or six minutes; but a curious feature in the behavior of these eggs is that they remain smooth and spherical during the entire period of shrinkage, the surface showing no sign of the folds and crenations characteristic of the fertilized eggs. It is evident that as a result of fertilization the properties of the plasma-membrane have undergone profound alteration affecting both its osmotic properties and its physical consistency, so that this simple osmotic treatment is sufficient to differentiate sharply between the two kinds of eggs.

An incidental effect of the above treatment is that the rapid abstraction of water from the fertilized eggs causes a considerable increase in their density, hence they sink in the hypertonic sea-water more rapidly than the unfertilized eggs; in fact a partial separation of the two kinds can readily be accomplished by taking advantage of this difference in the rate of sinking. The following experiment will illustrate. To a mixture of fertilized and unfertilized eggs in normal sea-water a relatively large volume of hypertonic van't Hoff's solution (1m; O.P. = *ca.* 40 atm.) was added; the eggs were then uniformly distributed in a graduate and allowed to sink. At the end of three minutes and again at the end of twelve minutes some eggs were removed with a pipette from the upper layer of the sinking mass, and the proportions of fertilized and unfertilized were determined by counting the numbers of each kind in several microscopic fields under the low power. The numbers obtained were as follows:

	FERTILIZED	UN-FERTILIZED
Before sinking	48	70
After 3 minutes sinking (sum of four counts)	46	89
After 12 minutes sinking (sum of eight counts)	17	116

It is evident that the proportion of unfertilized eggs in the surface layer increases rapidly as the eggs sink through the solution. A practical method of separating fertilized from unfertilized eggs without injury—in case such a method were required for any purpose—could no doubt be devised, e.g., by using moderately hypertonic sea-water and a centrifuge.

When eggs that have been shrunken in hypertonic sea-water are returned to normal sea-water, water reënters the fertilized eggs rapidly, the unfertilized eggs slowly. It has already been shown that shrinkage in moderately hypertonic sea-water does not impair the developmental capacity of the eggs; and correspondingly the osmotic properties of the plasma-membranes appear to be unaffected by this treatment, as indicated by the following experiment. Mixed fertilized and unfertilized eggs were placed in hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 4 vols. sea-water) and left for five minutes; at this time the fertilized eggs had the typical collapsed and angular appearance and were smaller than the round unfertilized eggs which had not yet reached osmotic equilibrium. The eggs were then returned to normal sea-water. After one minute in sea-water the fertilized eggs were again round and already distinctly larger than the unfertilized eggs—showing that even under a smaller gradient of osmotic pressure water reenters the fertilized eggs more rapidly. After two or three minutes in sea-water the difference in size was still apparent though less so than before; later, as the unfertilized eggs also approached osmotic equilibrium, the difference disappeared. It is clear that the resistance to the passage of water in either direction through the plasma-membrane is much smaller in fertilized than in unfertilized eggs. This experiment thus yields the same result as the earlier experiments in which eggs were transferred from normal to dilute sea-water—in this case also from a more to a less concentrated medium. The use of dilute sea-water in the osmotic method of determining relative permeability has the advantage that a quantitative comparison between the rates of entrance of water is possible, since the eggs remain spherical and the volumes can be calculated from the diameters; this is not possible when hypertonic sea-water is used, because of the irregular form of the collapsed fertilized eggs.

The rapid dehydration in strongly hypertonic solutions has a destructive action upon fertilized eggs (see table 1); unfertilized eggs, in correspondence with their slower shrinkage, are less injured by such solutions, as is shown by the following experiment. Equal quantities

of fertilized and unfertilized eggs in separate watch-glasses were exposed to equal volumes of strongly hypertonic sea-water (10 cc. of a mixture of 10 vols. 2.5m van't Hoff's solution *plus* 15 vols. sea-water). Within one minute the fertilized eggs turned pink in color, a sign of cytolysis, and within two minutes the solution was deeply colored with escaped pigment. After the same interval the unfertilized eggs showed no indication of cytolysis and the solution remained clear; an hour later the eggs were still uncytolyzed, although much shrunken, and there was no evident loss of pigment. Loeb and others have described experiments showing the greater resistance of unfertilized eggs to various forms of toxic action;[14] and the above observations indicate that this difference depends primarily upon the difference in the properties of the semi-permeable protoplasmic surface-layer (or plasma-membrane) before and after fertilization. The precise physico-chemical basis of this difference remains to be determined; there are, however, two definite changes in the physical properties of the plasma-membrane which are brought out clearly by the above experiments: (1) the increased permeability to water following fertilization, i.e., decreased resistance to its passage or decreased waterproof property, and (2) the simultaneous disappearance of the tension or contractile properties of the surface-film of protoplasm. The decrease in the resistance to toxic agents is apparently correlated with this change of properties in the membrane.

The latter of these two changes is remarkable. In the unfertilized egg, as already described, the surface contracts or decreases in area as water is lost, so that the whole egg remains smooth and spherical—indicating the existence of a certain contractile tendency or tension in the surface-film; i.e., the surface acts as if fluid or elastic in its properties, while in the fertilized egg even a slight decrease in volume throws the surface into folds, indicating the lack of any such effective surface-tension. Apparently the surface-film in the latter case is solid and under little or no tension; hence its area does not adjust itself to the decreased volume of the egg. It should be noted here that certain constant differences in the mechanical and other properties of the egg-surface before and after fertilization have also been observed by Chambers,[15] using the methods of microdissection. His observations, not yet published in detail, should be correlated with those above described.

The action of hypertonic sea-water upon eggs with artificial fertilization-membranes. Eggs in which artificial fertilization-membranes have been

[14] J. Loeb: Biochem. Zeitschr., 1906, i, 200; 1906, ii, 81.

[15] See the footnote on p. 264 of my former paper, *loc. cit.*

formed by exposure to butyric acid solution exhibit the same increase of water-permeability and liability to crenation as sperm-fertilized eggs; but the effects are more variable and present an interesting series of gradations. The following description of a typical experiment will illustrate:

August 17, 1917, Unfertilized Arbacia eggs were exposed for seventy-five seconds to a solution of N/260 butyric acid in sea-water (2 cc. n/10 acid *plus* 50 cc. sea-water) and then returned to normal sea-water. Twenty-five minutes later a strongly hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 2 vols. sea-water) was added to a mass of these eggs in a watch-glass (lot A). At the same time, for comparison and control, a similar quantity of sperm-fertilized eggs (fertilized twenty-five minutes previously) was similarly treated in a second watch-glass (lot B).

In lot A the majority of eggs showed well-marked shrinkage and crenation in less than one minute. The shrinkage was however on the whole less marked than in lot B and its degree was more variable; a considerable proportion of eggs in which membranes were imperfectly separated or absent shrank slowly and remained round; even after three minutes this minority (*ca.* 20 to 30 per cent) were still rounded and less shrunken than the others. In general the eggs with the most definite and well-separated membranes showed the most rapid and complete shrinkage and crenation. There was also a considerable loss of pigment from the eggs in lot A, but distinctly less than in lot B; closer examination showed that in the eggs with imperfect membranes or without visibly separated membranes the cytolytic effect was slight or absent.

In the control experiment (lot B) all eggs underwent rapid and complete collapse and the loss of pigment was well-marked.

Several other experiments of the same kind, with less strongly hypertonic solutions, gave the same general result. It is well known that artificial membrane-formation in Arbacia eggs is a highly variable process and that the membranes are typically thinner than those formed in normal fertilization and adhere more closely to the egg-surface; usually in a minority of eggs no separate membrane can be seen. In all cases it was found that those eggs which underwent the most rapid and well-marked collapse were those having well separated and definite membranes; eggs with imperfect membranes shrank more slowly and tended to remain round; while there was always present a certain variable proportion of eggs, without visibly separated membranes, which exhibited a behavior almost indistinguishable from that of unfertilized eggs, shrinking slowly and remaining quite round.

A definite correlation was thus found between the degree of membrane-separation and the degree of increase in permeability to water. The eggs with the most nearly normal membranes approach most

nearly in their osmotic behavior to sperm-fertilized eggs. It seems probable that the degree of completeness of the activation-effect is similarly determined; as a rule the results of artificial parthenogenesis in Arbacia show wide variation and only a small proportion of eggs form normal larvae. I have not yet investigated the effect of the second "corrective" treatment with hypertonic sea-water upon the water-permeability of these eggs. It is clear, however, that the membrane-forming agent, when effective, produces the same kind of effect upon permeability as the normal entrance of the sperm. This may also be demonstrated by the use of dilute sea-water; eggs with artificial membranes swell more rapidly than normal unfertilized eggs, although on the whole less rapidly and at a more variable rate than sperm-fertilized eggs.[16]

Effects of hypertonic sea-water upon Echinarachnius eggs. Experiments similar to the above were performed with the fertilized and unfertilized eggs of the sand-dollar, Echinarachnius parma. These eggs are spherical in form and much larger than those of Arbacia, having an average diameter of about 140μ;[17] they have a clear protoplasm, only slightly pigmented, and are highly favorable objects for experimental purposes. They are readily obtained in quantity and resemble sea-urchin eggs in their general properties.

Unfertilized eggs placed in a strongly hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 2 vols. sea-water) shrink gradually and remain round, while fertilized eggs in the same solution shrink rapidly and crenate. A mixture of fertilized and unfertilized eggs exhibits the same kind of contrast, after a minute or two in the solution, as Arbacia eggs. This contrast is most striking in about two minutes; fertilized eggs also sink more rapidly in the solution than unfertilized eggs.

Similar experiments with a concentrated van't Hoff's solution (1.25m) gave the same result. After one minute in this solution the fertilized eggs are collapsed and crenated and smaller in diameter than the unfertilized eggs which shrink slowly and remain round. If such mixed eggs are returned to normal sea-water, after four or five minutes' exposure to the hypertonic solution, the fertilized eggs regain water much more rapidly and within a minute are round and larger than

[16] See the curve representing the average rate of intake of water by these eggs in hypotonic sea-water on p. 255 of my former paper (*loc. cit.*).

[17] The average diameter of 18 unfertilized Echinarachnius eggs, measured with the ocular micrometer, was 139.4μ. Arbacia eggs measure about 74μ.

the unfertilized eggs. As in the similar experiment with Arbacia eggs, this difference is temporary and disappears as the eggs near osmotic equilibrium. Fertilized eggs also swell more rapidly in dilute sea-water. Experiments with artificially activated eggs have not yet been made.

Starfish eggs. Experiments with starfish eggs have been few in number as yet, but a brief report seems desirable since an entirely different type of behavior was found. These eggs are usually difficult to procure at the time of year (late August) in which these experiments were begun. Only one normal lot was obtained but these showed in hypertonic sea-water a definite behavior which is undoubtedly characteristic. Unfertilized mature eggs were found to shrink in concentrated sea-water much more rapidly than the eggs of either Arbacia or Echinarachnius, and fertilization had little or no effect upon the rate or character of shrinkage. It was also found that fertilized and unfertilized eggs swelled in dilute sea-water at about the same rate. These observations indicate that in the mature unfertilized starfish egg the permeability to water is already relatively high and undergoes little or no change as a result of fertilization. Further investigation of the conditions in these eggs is, however, desirable; and the present incomplete results are cited chiefly because of the suggestive parallel which they show to the observations of Loeb and Wasteneys[18] upon the relative rates of oxidation before and after fertilization. In the starfish egg these investigators found no significant difference; while in the Arbacia egg fertilization increased the rate of oxygen-consumption nearly four-fold, i.e., to about the same degree as the permeability to water. The absence of increase in water-permeability in the starfish egg has probably some direct relation to the absence of increase in oxidations. In both species of eggs the rate of oxygen-consumption appears to run parallel with the permeability to water.

III. TIME-RELATIONS OF THE INCREASE OF PERMEABILITY IN ARBACIA EGGS

The change of permeability following fertilization in Arbacia eggs is not sudden or rapid but begins gradually and requires a considerable time—at least twenty minutes at the summer temperature of the sea-water (20 to 22°)—to reach approximate completion. During the first few minutes after insemination the eggs, when placed in hypertonic

[18] J. Loeb: Artificial parthenogenesis and fertilization, Univ. of Chicago Press, 1913, chapter II.

sea-water, shrink slowly and remain round, like unfertilized eggs. Not until five or six minutes have elapsed is there any noticeable increase in the permeability to water, as indicated by increased rate of shrinkage; from then on the rate becomes by degrees more and more rapid and the tendency to crenation also appears and increases *pari passu* with the rate of shrinkage. Both of these effects are clearly expressions of the same change in the plasma-membrane. In about twenty minutes the process of permeability-increase is nearly complete; but usually a test with hypertonic sea-water made at thirty or forty minutes after insemination shows a distinctly greater rate and degree of collapse than at twenty minutes, indicating that the membrane continues to change slowly for some time afterwards. The condition of increased water-permeability appears to remain as a permanent property of the developing egg, and may readily be demonstrated in the two-cell and four-cell stage in the intervals between cleavages; such eggs collapse rapidly and crenate in the same manner as the uncleaved egg.[19]

The general course of the process can best be indicated by the description of a typical experiment in table 2. This summarizes a series of observations in which eggs from a single fertilized lot were tested in hypertonic sea-water (1 vol. 2.5m van't Hoff's solution *plus* 4 vols. sea-water) at successive intervals of two minutes through a total period of sixteen minutes, beginning immediately after insemination. Each portion of eggs was examined immediately after placing in the solution and again later after nineteen minutes in the solution.

Several other similar series gave the same general result. It will be seen that the differences between the successive members of such a series are easily appreciable at first, but become less so later. Apparently the increase of permeability begins between two and four minutes after insemination and is in greater part completed during the next ten minutes. The process continues long after the complete separation of the fertilization-membrane and cannot be referred directly to the formation of this structure or to any changes in its properties after separation. Heilbrunn has shown that the fertilization-membrane undergoes an increase of permeability to salts during the first few minutes after separation;[20] but its prompt collapse in sea-water con-

[19] According to J. Gray (personal communication) the electrical conductivity of Echinus eggs after fertilization remains permanently greater than that of unfertilized eggs (*cf.* my recent paper on the present subject, this Journal, 1917, xliii, footnote p. 44).

[20] Heilbrunn: Biol. Bull., 1915, xxix, 160.

taining a little egg-albumin (which raises osmotic pressure very slightly) shows that it offers only a negligible resistance to the passage of water. Moreover this change of permeability is completed within five minutes,[21] i.e., at a time when the permeability of the egg to water is just

TABLE 2

TIME OF PLACING IN SOLUTION (MINUTES AFTER FERTILIZATION)	EFFECTS OF EXPOSURE TO HYPERTONIC SOLUTION
1. 2m	Fertilization-membranes are well separated in all eggs. All remain round without any crenation and shrink slowly. At 19 minutes all are round, shrunken and crenated
2. 4m	After 1 minute nearly all eggs are round and not evidently shrunken, but a few show traces of crenation. At 19 minutes the great majority are round and shrunken with smooth contour
3. 6m.	At 30 seconds all eggs are round; at 40 seconds a few are slightly crenated; at 1 minute most are moderately crenated but some remain round. At 19 minutes most eggs are again round, but the contours are slightly irregular in many
4. 8m.	Many eggs show slight crenation by 30 seconds; at 1 minute all are more or less shrunken and crenated. At 19 minutes nearly all show a slightly crenated or wavy contour; a few are round
5. 10m.	Most eggs show slight crenation at 20 seconds, well-marked at 30 seconds, and decided at 45 seconds and 1 minute. At 19 minutes all are more or less crenated and collapsed, but less so than in the later members of the series.
6. 12m.	Crenation is more rapid than in no. 5 and all eggs are well collapsed at 1 minute. At 19 minutes the degree of crenation is distinctly greater than in no. 5, and the eggs are collapsed and polygonal in form
7. 14m.	Crenation is rapid and pronounced in all eggs, more so than in no. 6. At 19 minutes the degree of collapse and irregularity of form appears somewhat greater than in no. 6, and rounded eggs are fewer
8. 16m.	Crenation and shrinkage are rapid and pronounced, as in no. 7, but with no evident difference. At 19 minutes the condition is the same as in no. 7.

beginning to show increase, hence it cannot be regarded as a factor of any importance in the above effects. There seems to be no doubt that a progressive change in the osmotic properties of the semi-permeable surface-layer of the egg-protoplasm (the plasma-membrane proper) is

[21] Heilbrunn: *loc. cit.*, 161.

chiefly or wholly responsible for the change in the behavior of the egg in the anisotonic medium. This change is only one of many features in the complex of reactions initiated in the egg by the entrance of the spermatozoön, and presumably it is to be referred to some special metabolic process taking place in the surface-film. It is interesting to note that the period of permeability-increase shows a general correspondence in its time-relations with the period of increased susceptibility to poisons which, as shown by Lyon,[22] immediately succeeds fertilization. At this time there appears to be a well-marked increase in the general rate of metabolism, as indicated both by the increased output of CO_2 and by the greater susceptibility to cyanide;[23] and this increase in metabolism is probably directly connected with the change in the properties of the membrane.

IV. INFLUENCE OF EXTERNAL CONDITIONS ON THE CHANGE OF PERMEABILITY

Experiments on the influence of external conditions upon the change of permeability in Arbacia eggs have shown that the process is not readily affected by increasing the calcium-content of the sea-water or by low concentrations of cyanide. Since these conditions both inhibit cleavage, a further indication is afforded that the physical or metabolic change in the plasma-membrane underlying the increase of permeability is of a special kind and different from that associated with cytoplasmic division, where also a demonstrable change in the properties of the membrane takes place.[24] On the other hand, the process is checked or arrested reversibly by higher concentrations of cyanide (M/200 and above), and also by anaesthetics. The influence of temperature has not been studied as yet.

[22] Lyon: This Journal, 1902, vii, 56.

[23] For increased output of CO_2 by Arbacia eggs after fertilization *cf.* Lyon: This Journal, 1904, xi, 52. The work of Child, in collaboration with Tashiro, indicates that the degree of susceptibility of cells to cyanide is a general index of the rate of metabolism (as measured by output of CO_2). *Cf.* Child: Senescence and rejuvenescence, Univ. of Chicago Press, 1915, chapter iii.

[24] During the formation of the cleavage-furrow the plasma-membrane loses its resistance to disruption in dilute sea-water, and the eggs then rapidly break down in this medium, recovering their resistance after cleavage is complete. There is no such decrease in the resistance to osmotic disruption immediately after fertilization—a fact indicating that the change then taking place in the plasma membrane is of a different kind from that associated with cleavage. *Cf.* Journ. Exper. Zoöl., 1916, xxi, 369; see 386.

It was expected that a decided increase in the calcium-content of the sea-water would retard or prevent the change of permeability, but this was found not to be the case. Eggs placed, two minutes after insemination, in a mixture of equal volumes isotonic $CaCl_2$ (0.35m) and sea-water were found after sixteen minutes in this solution to shrink and crenate promptly in hypertonic sea-water, showing little or no difference from eggs similarly treated after an equal interval in normal sea-water. The change of permeability thus proceeds at an unaltered rate in the presence of a high concentration of calcium.

The change is also not noticeably influenced by low concentrations of KCN, although high concentrations are effective. Eggs placed, two minutes after insemination, in sea-water containing M/1000 KCN were found to undergo rapid shrinkage and crenation on transfer to hypertonic sea-water sixteen minutes later; no difference from the control could be seen. For the complete prevention of cleavage, on the other hand, M/8000 KCN is sufficient.[25] Experiments with higher concentrations of KCN (M/800, M/400, M/200, M/100) gave a different result; eggs were placed in these solutions two or three minutes after fertilization, and after thirty to thirty-five minutes exposure were examined in hypertonic sea-water. After exposure to M/100 and M/200 KCN shrinkage was gradual and only a small proportion of eggs underwent partial crenation; with M/400 KCN shrinkage was more rapid and considerable crenation took place, indicating retardation but not entire prevention of the change of permeability; while with M/800 KCN the behavior was indistinguishable from the control. This inhibiting action of cyanide is reversible; eggs replaced in normal sea-water after the above exposures all showed thirty minutes later the typical rapid shrinkage and crenation. The replaced eggs of these lots left undisturbed in sea-water almost all developed to larval stages.

Organic anaesthetics readily inhibit the increase of permeability and the effective concentrations were found for the most part similar to those required for the prevention of cleavage in these eggs,[26] although in several instances they were somewhat higher. Experiments were made with chloral hydrate, chloroform, methyl, ethyl, propyl, isobutyl and iso-amyl alcohols, ethyl urethane and ethyl ether. In all cases

[25] *Cf.* Journ. Biol. Chem., 1914, xvii, 121; see 137.

[26] Journ. Biol. Chem., *loc. cit.* Similar concentrations of anaesthetic inhibit membrane-formation and activation in Arbacia eggs by pure isotonic solutions of neutral salts (*cf.* Journ. Exper. Zoöl., 1914, xiv, 591); they also retard the cytolytic action of such solutions on unfertilized eggs (*cf.* this Journal, 1912, xxx, 1).

eggs which were placed, two or three minutes after insemination, in solutions of these compounds in sea-water, of the appropriate concentrations, remained in the characteristic water-impermeable and slowly shrinking condition during the period of exposure to the anaesthetic, or underwent only slight and gradual change. On return to normal sea-water the permeability-increasing process was resumed and the eggs continued development, nearly all reaching larval stages.

The following description of experiments with solutions of chloral hydrate in sea-water is typical of the procedure and results with the above anaesthetics. The concentrations of chloral hydrate employed were 0.3, 0.2 and 0.1 per cent. The eggs were placed, three minutes after insemination, in the solutions, and left for thirty to thirty-five minutes; they were then transferred directly to hypertonic sea-water and the behavior was observed. In 0.3 per cent chloral hydrate the change of permeability appeared to be entirely prevented; one and a half minutes after placing in the hypertonic sea-water the eggs were still round, uncrenated and only slightly shrunken; ten minutes later shrinkage was well-marked but the contour remained smooth, as in the case of unfertilized eggs. Part of the eggs were then transferred from the anaesthetic solution to normal sea-water and thirty minutes later the reaction to hypertonic sea-water was again tested; rapid shrinkage and crenation were then found, showing that the process of permeability-increase had been resumed after the removal of the anaesthetic. Eggs that were left undisturbed in normal sea-water, after return from the anaesthetic solution, continued development and the great majority formed larvae. Similar results were obtained with 0.2 per cent chloral hydrate, but with the 0.1 per cent solution the increase of permeability, though retarded, was not entirely prevented; the eggs shrank in the hypertonic sea-water more slowly than the control unanaesthetized eggs, but more rapidly than eggs that had been treated with the two stronger solutions, and many showed partial crenation. The concentrations required for complete prevention of permeability-increase are thus higher than for the prevention of cleavage; the latter concentration is about 0.1 per cent in Arbacia eggs.

With all of the other anaesthetics results of a similar kind were obtained; the change of permeability was either prevented or markedly retarded in the anaesthetic-containing sea-water, and renewed on return to normal sea-water, and the returned eggs continued development, the great majority forming larvae. In most cases a reversible arrest or a marked retardation was found in solutions which were just

concentrated enough to prevent cleavage, but in some other cases (ether, amyl alcohol) somewhat stronger solutions were needed. The following table gives the concentrations of the solutions in which increase of water-permeability is completely or almost completely arrested without injury to the eggs. The concentrations required to anaesthetize cleavage are also given for comparison. Solutions only slightly weaker than these retard without preventing the process, or in some cases they have little or no evident action; e.g., in 0.5 vol. per cent amyl alcohol or 1 vol. per cent ether the increase of permeability takes place at almost the normal rate.

TABLE 3

ANAESTHETIC	CONCENTRATION PREVENTING INCREASE OF PERMEABILITY	CONCENTRATION PREVENTING CLEAVAGE[27]
Chloral hydrate	*ca.* 0.2 per cent	0.1–0.2 per cent
Chloroform.....	$\frac{1}{16}$ saturated (0.05 per cent)	$\frac{1}{12}$ saturated (0.06 per cent)
Methyl alcohol	8 vol. per cent	
Ethyl alcohol	5 vol. per cent	5 vol. per cent
n-Propyl alcohol	2 vol. per cent (effect is incomplete in some eggs)	2 vol. per cent
Isobutyl alcohol	1–1.2 vol. per cent	0.8 vol. per cent (for n-butyl alcohol)
i-Amyl alcohol	0.6 vol. per cent (0.5 vol. per cent is insufficient)	*ca.* 0.4 vol. per cent
Ethyl urethane	2 per cent	1.5–1.75 per cent
Ethyl ether	1.2–1.4 vol. per cent (1 vol. per cent is insufficient)	0.5–0.6 vol. per cent

The general correspondence of the above two series of concentrations shows that the process determining the permeability-increase of fertilization is anaesthetized under essentially the same conditions as that determining cell-division. In both cases a change normally taking place in the physical properties of the protoplasmic surface-layer—shown by increase of water-permeability in the one case, and by loss of resistance to osmotic disruption in the other—is prevented. The theory that anaesthetic action consists essentially in a modification in the state of the "plasma-membrane"—the general name for the water-insoluble and semi-permeable surface-film of protoplasm—is thus favored. It should however also be noted that the higher concentrations of anaesthetic required to arrest the former process in several instances, as well as its greater resistance to cyanide, indicate that the underlying

[27] Journ. Biol. Chem., *loc. cit.*

physico-chemical conditions are not identical in the two cases.[28] Apparently variations in the permeability and other properties of the plasma-membrane may take place as the result of more than one kind of change in this structure.

In general anaesthesia appears to be associated with an increased stability of the plasma-membrane and a correspondingly increased resistance to change of permeability and hence of electrical polarization.[29] The membrane preserves its semi-permeability under conditions which in the unanaesthetized cell cause temporary or permanent loss of this property; hence the greater resistance to certain forms of cytolytic action as well as the lack of response to stimulation.[30] There may also be an actual decrease in the normal resting permeability of the cell; this has been observed in several cases; for example, the electrical conductivity of plant-cells may be decreased by 13 per cent during anaesthesia, as observed by Osterhout in Laminaria.[31] I have also found that after the normal increase of permeability to water is completed in fertilized Arbacia eggs, it is possible to cause a return toward a condition of decreased permeability by exposure to the above solutions of anaesthetics. This is shown by the following experiments. The eggs were placed, about thirty minutes after fertilization, in the anaesthetic-containing sea-water, and after remaining in this solution for twenty to thirty minutes the osmotic behavior in hypertonic sea-water (1 vol. 2.5m van't Hoff's sol. *plus* 4 vols. sea-water containing the same anaesthetic in the same concentration) was tested in the usual manner. The following table summarizes the results of several observations with each anaesthetic.

These observations show that the abstraction of water from fertilized eggs by hypertonic sea-water is definitely retarded after exposure to nearly every one of the above anaesthetic solutions, in some cases markedly, in others comparatively slightly. Cyanide proved ineffective. The greatest permeability-decreasing action was shown by ethyl urethane; eggs treated with this compound remain round and uncrenated for several minutes after placing in the hypertonic sea-water, and

[28] *Cf.* footnote 24.

[29] The evidence for this general theory is summarized and discussed in my article on "The theory of anaesthesia:" American Yearbook of Anaesthesia, 1916; also in Biol. Bull., 1916, xxx, 311.

[30] *Cf.* the general article just cited; Biol. Bull., pp. 356. *seq.* also footnote 26 above.

[31] Osterhout: Science, N. S., 1913, xxxvii, 111.

TABLE 4

ANAESTHETIC AND CONCENTRATION	RESULTS OF EXPOSURE TO HYPERTONIC SEA-WATER
1. KCN, M/200	Rapid shrinkage and crenation as in the control
2. Chloral hydrate	
(a) 0.3 per cent	Distinct decrease in the rate of shrinkage. After 2 minutes in hypertonic sea-water the eggs remain almost round and are much less shrunken than in the untreated eggs of the control, which are all typically shrunken and collapsed in less than 1 minute
(b) 0.15 per cent	Some retardation of shrinkage but much less than in (a)
3. Chloroform $\frac{1}{8}$ and $\frac{1}{10}$ saturated°	Here there was no evident effect
4. Methyl alcohol 8 vol. per cent	Crenation and shrinkage are decidedly retarded; most eggs remain round and relatively slightly shrunken after 2 minutes in the hypertonic sea-water
5. Ethyl alcohol 5 vol. per cent	Also distinct retardation of shrinkage; after 1 minute crenation is slight and many eggs are still round
6. Propyl alcohol 2 vol. per cent	Shrinkage and crenation are retarded, but not markedly; after 1 minute the eggs are slightly or moderately crenated
7. Isobutyl alcohol	
(a) 1.4 vol. per cent	Shrinkage and crenation are distinctly retarded; after 1 minute many eggs remain round and only slightly shrunken; others are more shrunken and slightly or moderately crenated
(b and c) 1 and 1.2 vol. per cent	Also some evident retardation but less than in (a).
8. i-Amyl alcohol	
(a) 0.6 vol. per cent	Shrinkage and crenation are somewhat retarded but not markedly
(b) 0.5 vol. per cent	No evident retardation in this solution
9. Ethyl urethane 2 per cent	Rate of shrinkage is greatly decreased; after 1 minute in the hypertonic sea-water all eggs remain round and uncrenated
10. Ethyl ether from 1.2 to 0.5 vol. per cent	Some slight retardation was observed in the 1.2 per cent solution; in the weaker solutions (1.0, 0.8, 0.75, 0.5 vol. per cent) there was no evident effect

later, after shrinkage is completed, exhibit only slight crenation. In fertilized eggs thus completely anaesthetized the plasma-membrane resembles that of unfertilized eggs in its general properties although the degree of impermeability is not so great. Chloral hydrate and the alcohols also cause well-marked decrease of permeability to water, but

with ether and chloroform the effect was slight or lacking. In all cases the reversibility of the change induced by the anaesthetic was proved by subjecting the eggs to a second osmotic test about twenty minutes after returning to sea-water; the tested eggs then showed the typical rapid collapse and crenation, and eggs left undisturbed after the return to sea-water continued their development to larval stages.

It is clear that the anaesthetic modifies the permeability of the membrane to water as well as its general stability or resistance to alteration.[32] Just how this effect is produced remains for the present problematical. The anaesthetic may promote the continuity of the non-aqueous or lipoid phase of the protoplasmic emulsion at the boundary-surface of the cell, possibly in the manner suggested by Clowes;[33] or it is possible that by dissolving in this phase it may increase the relative volume occupied by the colloidal particles of lipoid and hence decrease the relative volume of the aqueous phase of the emulsion, thus making the latter a more effective barrier to the passage of water (and presumably to water-soluble substances also). In all probability the total effect depends upon a combination of several distinct actions, in which metabolic factors also enter. The plasma-membrane is undoubtedly the seat of an active metabolism of an oxidative type, and substances produced, altered or destroyed in this metabolism must influence its physical and other properties.

SUMMARY

1. Fertilized eggs of Arbacia and Echinarachnius shrink rapidly and undergo crenation in hypertonic sea-water or van't Hoff's solution (of 30 to 40 atmospheres O.P.); unfertilized eggs shrink slowly in the same solutions and remain round. The relative rates of swelling in dilute sea-water are similar. Fertilization thus results in a marked increase in the permeability of the plasma-membrane (the semi-permeable surface-layer of protoplasm) to water.

2. Artificial formation of fertilization-membranes by butyric acid causes similar though more variable effects in Arbacia eggs. Eggs with

[32] It has been mentioned that anaesthetized uncleaved eggs show greater resistance than normal eggs to the permeability-increasing action of pure isotonic salt-solutions; this fact explains why the activating effect of such solutions is prevented by anaesthetics, since activation involves increase of permeability; similarly with the inhibiting action of anaesthetics on cleavage which also is associated with a change in the plasma-membrane.

[33] Clowes: Journ. Phys. Chem., 1916, xx, 407.

well-separated membrane exhibit a well-marked increase of permeability like that of sperm-fertilized eggs; when membrane-formation is imperfect or lacking the increase of permeability is less marked or may not be evident.

3. The change of permeability is a gradual process, beginning between two and four minutes after insemination and reaching an approximate final stage in about twenty minutes (at 20 to 22°).

4. The change of permeability is arrested or retarded reversibly by potassium cyanide in concentrations of M/100 to M/400; concentrations of M/800 and lower are ineffective. This change is much more resistant to cyanide than cleavage.

5. Anaesthetics (chloral hydrate, alcohols, urethane, ether) also prevent the increase of permeability, in concentrations which are similar to but in some cases higher than the concentrations arresting cleavage in the same eggs. The effect is readily reversible. A direct influence of anaesthetics upon the permeability to water is thus demonstrable. Eggs which have undergone the normal increase of permeability following fertilization also show a reversible decrease of permeability to water in solutions of certain anaesthetics (chloral hydrate, alcohols, urethane).

AN EXPERIMENTAL STUDY OF ALTERNATING GROWTH AND SUPPRESSION OF GROWTH IN THE ALBINO MOUSE, WITH SPECIAL REFERENCE TO THE ECONOMY OF FOOD CONSUMPTION[1]

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INTRODUCTION

Although much is known regarding the conditions under which growth may be retarded or promoted, the food consumption in relation to growth has not been studied extensively. For this reason it was thought that an investigation of the economy of food in alternating periods of growth and suppression of growth in an animal for which the diet could be controlled with accuracy would prove to be of value. The white mouse was chosen for study in the present research. It was planned to make the comparative food intake during normal, suppressed and accelerated growth the basis for a consideration of the economy of food in growth as a contribution to some of the problems of animal production. Statistics are herewith offered for the average daily food requirement, together with the curves of normal growth for both male and female mice from the age of weaning, 22 days, to the age at which average adult weight is reached, 62 days. The food consumption per day of normally growing mice has been estimated for varying body weights of from 7 to 25 grams. The daily food requirement for maintaining body weight both in an initial suppression and in repeated suppressions of growth has been compared with that for normal growth. The total food required to complete growth during a period of refeeding after suppression of growth has been compared with the food consumption of controls making the same growth from

[1] This study was aided by a grant from the Elizabeth Thompson Science Fund. The data are taken from the dissertation presented by Helen B. Thompson for the degree of Ph.D., Yale University, 1917.

the same initial weight. The total food consumed during the period of suppression of growth and the following period of refeeding has also been compared with the total consumption of the control animals for an equal number of days at corresponding ages.

It was hoped by the study indicated above to test the following points: the relation of the food intake to normal growth at different ages and at varying body weights; the food requirement per day in suppressed growth for varying periods and for successive suppressions; the economy of food in accelerated growth; the cost, in total food, of maintenance and growth when growth is completed after one or more suppressions.

PLAN OF INVESTIGATION

The mice were placed, when 20 to 22 days of age, in individual cages made of galvanized wire cloth. Absorbent paper covered with a mat of galvanized fly screen wire was used to line the bottom of the cage. The entire cage was cleaned at least once a week. Every day, while each mouse was being weighed before feeding, its cage was taken apart and the uneaten food collected, the feces removed and clean paper supplied if needed. Food and water cups were sterilized by boiling twice a week. Water cups were washed every day.

As a fairly high uniform temperature has been shown to be important in maintaining underfed animals in good health, the room temperature was kept day and night between 70° and 85°F.

The food was made up from the formula employed by Wheeler ('13) and later by Judson ('16).[2] This ration was selected because it had been demonstrated by Osborne and Mendel in feeding rats that a paste is desirable from the standpoint of economy in handling. It was essential to have a food that could be easily weighed out and from which refuse and scattered remnants could be accurately collected.

The food was made up as follows:

	grams
Skimmed milk powder	20
Casein	24
Starch	20

[2] Dissertation presented by S. E. Judson for the degree of Ph.D., Yale University, 1916. See Mendel and Judson: Proceedings of the National Academy of Sciences, 1916, ii, 692.

Salt mixture[3]	4
Butter fat	32

By analysis of a mixed sample of food prepared at several different times the composition was found to be:

	per cent
Protein (N x 6.25)	31.0
Fat (ether extract)	29.9
Carbohydrate	30.1
Ash	4.5
Water	4.5

Food was mixed fresh two or three times a week in quantities of 300 to 400 grams. It kept well without apparent fermentation or mold formation. Daily rations were weighed in grams and tenth grams; residues in milligrams.

In the early experiments it was noticed that a number of the mice ate irregularly and grew slowly. Those that did eat regularly grew at the rate described by Judson. As abnormally slow growth may indicate a low plane of nutrition brought about through failure of appetite as well as a deficiency in any food constituent, a source of vitamine in the form of 2 per cent of yeast was added to the diet without otherwise modifying the food mixture. This change was made for both the control mice and those maintained at constant weight. All animals ate much more regularly after the inclusion of yeast in the diet.

The yeast was "Torula" from the Hinckel Brewery Company, Albany, N. Y., which states that "Torula" contains on an average:

	per cent
Crude protein	46.6
Fat	0.5
Carbohydrates	32.3
Fiber	5.8
Ash	6.6
Water	8.2

With 2 grams of yeast added to 100 grams of food the estimated composition of the mixture then became:

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[3] Röhmann's salt mixture.

	grams
$Ca_3(PO_4)_2$	10
K_2HPO_4	37
NaCl	20
Na-citrate	15
Mg-citrate	8
Fe-citrate	2
Ca-citrate	8

(From Osborne and Mendel: Carnegie Inst. of Washington, 1911, Publ. 156, I, 32.)

	per cent
Protein	31.3
Fat	29.3
Carbohydrate	30.2
Ash	4.5
Water	4.6

The energy value of the food calculated from the usual factors is approximately 5.1 Calories per gram.

Mice at the age of 22 days range from 8 to 12 grams in body weight. Litters and groups for the same experiment were selected to start at approximately the same age with no greater variation in size than would be encountered in a single litter. In most cases the experiment was closed for the group when, after refeeding, a majority of the males had grown to the weight of 20 to 21 grams and the females to 18 to 19 grams, corresponding to the weights of the control animals at 45 to 50 days of age. The control animals were kept for comparison even after their weights were fairly constant. Data from all suppression tests have been included in the averages for maintenance, as stunted mice that did not respond to refeeding seemed able to maintain their weight on about the same amount of food as the other mice. Averages for renewed growth after suppression were computed for groups showing similar rates of acceleration. Slowly growing mice were included in the control curves, as in this case it was desirable to report averages from large numbers of unlike rates of growth.

NORMAL GROWTH AND DAILY FOOD CONSUMPTION OF ALBINO MICE FROM THE AGES OF 22 TO 62 DAYS

White mice reach sexual maturity and adult proportions in form at the age of about two months. The period following this age is one of very slow growth requiring, according to Judson, at least 40 days to make a gain of only 2.5 grams in weight. For this reason it was decided to include in these studies the normal growth curve to the 62d day only. The number of animals kept beyond the 54th day is rather small, but those that were allowed to live maintained average weight for 80 days or longer.

The body weights for all males included in the averages from which the curve was drawn are recorded in table 1, those for females in table 2. Mice 66, 87 and 102 made very unusual growths, but any increment which their weights might give to the averages are fully offset by the slowly growing mice 4, 7 and 36. The resulting curve is

TABLE 1

Body weight at age indicated in days. Males

MOUSE NUMBER	AGE IN DAYS										
	22	26	29	32	34	39	43	50	54	62	70
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
4	8.6	10.0	12.9	13.6	14.5	16.5	17.5	18.6	18.1		
7	7.9	10.0	11.5	13.7	14.2	16.0	17.3	21.2	22.7	24.0	24.0
19	13.4	14.0	15.9	17.9	18.5	19.5	20.6				
28	10.0	12.3	15.1	17.2	17.5	19.7	20.6	21.3	21.0	20.9	
36	7.0	9.7	9.9	9.9	11.0	15.3	16.9	19.2			
49	11.0	13.5	15.0	16.9	17.5	19.6	20.4				
57	11.7	13.7	15.5	16.2	17.6	18.0	17.3	18.0			
66	9.0	13.5	16.0	18.5	20.2						
73	9.1	12.6	15.5	17.9	19.0	19.0	19.9	20.5			
80	10.2	14.0	16.6	17.9	18.4	19.5	20.3				
87	11.2	15.1	18.9	20.7	23.2	25.7	26.4	27.1	27.2		
102	10.0	10.8	14.8	17.9	20.3	24.5	26.4	27.0			
113	10.0	12.5	13.6	15.9	17.9	21.0	22.3	21.4			
116	9.3	11.5	13.4	15.6	17.0	18.7	20.0	21.0	21.7		
117	10.6	12.9	15.3	17.0	17.3	19.5	20.5	21.4			
Average..	9.9	12.4	14.7	16.4	17.6	19.5	20.5	21.5	22.1	22.4	24.0

TABLE 2

Body weight at age indicated in days. Females

MOUSE NUMBER	AGE IN DAYS										
	22	26	29	32	34	39	43	50	54	62	70
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
5	8.4	10.0	11.5	13.0	14.0	15.2	15.6	16.0	16.5		20.2
12	8.5	11.0	13.0	14.2	15.0	16.0	17.0	17.1	17.7 (52 days)	18.8	
22	10.0	15.0	16.1	17.3	17.1	18.4	19.2	19.8	20.1		
43	8.0	10.5	12.1	14.7	16.1	18.5	19.5	20.3			
47	10.0	13.6	15.3	16.1	17.5	18.2	19.0	20.1	20.1		
56	9.6	12.2	13.6	14.4	15.1	17.5	17.9	18.7	18.9		
58	10.0	13.6	14.6	15.0	16.0	16.7	17.1	17.0	18.2	19.7	
60	8.0	10.0	11.6	12.9	13.2	14.7	16.0	16.3	16.8	17.8	
69	10.3	13.0	15.0	17.5	18.7	19.7	20.0				
94	10.7	13.3	14.9	17.0	17.9	18.2	19.0	19.7	19.9		
111	9.5	11.5	12.9	14.0	15.1	16.0	17.6				
Average...	9.4	12.2	13.7	15.1	16.0	17.2	18.0	18.3	18.5	18.8	20 3

about what it would be if both extremes of weight were excluded. The weights of the females did not show such wide variations but the average curve for this group departs more from Judson's curve than does that of the group of males.

The body weights for the normal curves which Judson discussed are given in table 3. The average weights for the fifteen males and eleven females grown as controls in the present experiment are given for corresponding days and for convenient dates between in order to describe curves that show their degree of regularity. The differences in the curves are apparent in chart I.

TABLE 3

AGE OF MOUSE	BODY WEIGHTS			
	Males		Females	
	Judson	Thompson	Judson	Thompson
days	*grams*	*grams*	*grams*	*grams*
Newborn	1.5	1.5	1.5	1.5
5	3.0	3.3	3.0	3.3
12	6.0	6.7	6.0	6.7
22	9.0	9.9	8.2	9.4
26	12.0	12.4	10.0	12.2
29		14.7		13.7
32	15.0	16.4	12.8	15.1
34		17.6		16.0
39	18.0	19.5	15.0	17.2
43		20.5		18.0
50	21.0	21.5	17.0	18.3
54		22.1		18.5
62		22.4		18.8
80	24.0	24.0	20.6	20.2
100	25.0		21.5	

Up to the 26th day the actual gain per day is the same for males and females. After this date the females gain on an average of 0.5 gram per day until the 34th day, when they drop to a lower rate. The males continue to add an increment of from 0.5 to 0.8 gram per day to their weight until about the 40th day of life. The rate of increase in body weight over the preceding day, for females, declines from 3 per cent on the 32d day to 2 per cent on the 40th day and to 0.3 per cent on the 62d day. The males grow at the rate of 4 per cent on the 32d day, 2 per cent on the 40th day, and 0.5 per cent on the 62d day.

Food consumption in relation to body weight. Males weighing from 9

to 13 grams, at 22 to 26 days of age, eat more *per gram body weight* than do the females, but there is the same rate of increase in body weight for both. After the 26th day the males continue to grow at the same rate until about the 40th day. The females change to a slower rate. This change occurs at the time that a slight reduction in the food consumption per gram body weight takes place. From the 26th day on the males have a larger daily food consumption but the food per gram body weight

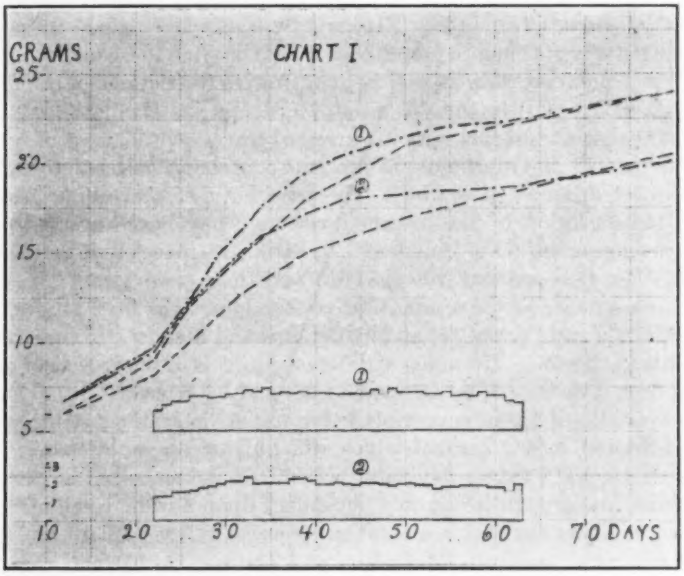

Chart I. Curve of normal growth and average daily food consumption. (1) Males (15 animals); (2) females (11 animals). - - - - Judson, -.-.-.- Thompson.

averages the same for both sexes until about the 40th day. After the 40th day the consumption per gram body weight of the males is from 0.1 to 0.2 gram in excess of that of the females. This slight excess furnishes the material for continuing growth, at the rate indicated by the curve, until about the 60th day. From the 60th day of life the curve for the males flattens and growth proceeds at the slower rate assumed by the females as early as the 40th day.

Table 4, giving maximum, minimum and average food consumption per day of normally growing mice from the ages of 22 to 62 days, and table 5, showing the food consumption per day at varying body weights, follow.

FOOD REQUIREMENT IN VARYING PERIODS OF SUPPRESSION OF GROWTH

The methods for suppressing growth applied by Röhmann ('08), Waters ('08), Aron ('10), Osborne and Mendel ('11–'17), Wheeler ('13), Jackson ('15), Judson ('16) and Stewart ('16) include the use of qualitatively inadequate proteins, exclusion of vitamines and limitation of the protein or salts, as well as quantitative restrictions of the daily intake of an otherwise suitable ration. Osborne and Mendel have found that the growth of rats may be arrested by the withdrawal of lysine from the diet and by the use of proteins containing less than the minimum requirement of cystine. The possibility of controlling growth by the exclusion of the so-called vitamine has been demonstrated by the investigations of Hopkins ('12), Funk ('12), McCollum and Davis ('13–'15), Osborne and Mendel ('13) and their coworkers. The protein requirement from a quantitative standpoint has been studied by McCollum and Davis ('15) and by Osborne and Mendel ('15) in experiments upon rats. The value of different proteins in the growth of mice has been investigated by Röhmann ('08) and by Wheeler ('13). From these studies it has been possible to formulate dietaries containing protein limited to the amount which will provide for maintenance but suspend growth for an indefinite period. It is recognized at present that normal growth is not only dependent upon a wide distribution of inorganic salts but that in animal nutrition as well as in plant the "law of minimum" applies. McCollum and Davis ('12–'15) and Osborne and Mendel ('13) have shown that the growth of rats may be checked by a limitation of the salts. Judson ('16) used this method successfully in suppressing the growth of mice.

Statistics of daily food consumption for mice held at constant body weights for 5, 9, 18 and 27 days respectively. The tests of food consumption in suppressed growth began with mice at 20 to 23 days of age. One litter of eleven was taken from the mother at the end of two weeks and fed on milk and the experimental food until 30 days old. As they had at that time barely reached the average size of the 22-day-old mice, they were started upon suppression tests. Their subsequent growth proved that they were developing at the normal rate for their size rather than

TABLE 4

Food consumption per day of normally growing mice at successive ages. Age 22–62 days

AGE	MALES				FEMALES			
	Number of animals	Food eaten			Number of animals	Food eaten		
		Maximu	Minimum	Average		Maximum	Minimum	Average
days		*grams*	*grams*	*grams*		*grams*	*grams*	*grams*
22	9	1.5	0.6	1.2	3	1.4	1.1	1.3
23	12	2.2	1.0	1.5	9	2.2	0.9	1.3
24	14	2.5	1.0	1.7	11	2.3	0.8	1.6
25	14	2.4	1.0	1.7	11	2.6	0.9	1.6
26	14	3.1	1.1	2.0	11	2.2	0.8	1.7
27	14	2.8	1.3	2.0	11	2.5	0.8	1.8
28	14	2.6	0.8	1.9	11	2.4	1.1	1.9
29	14	2.5	1.0	1.9	11	2.7	1.5	2.1
30	15	3.0	1.3	2.1	11	2.5	1.8	2.2
31	15	3.0	1.4	2.1	11	2.9	1.9	2.2
32	15	3.2	1.2	2.3	11	3.1	1.9	2.4
33	15	3.5	1.5	2.2	11	3.1	1.5	2.1
34	14	3.3	1.6	2.4	11	2.8	1.4	2.1
35	14	2.6	1.6	2.1	11	2.9	1.0	2.1
36	14	3.1	1.6	2.3	11	2.5	1.6	2.1
37	14	3.1	1.2	2.2	11	2.7	1.7	2.3
38	14	3.1	2.0	2.3	11	2.6	1.4	2.3
39	14	2.9	1.3	2.2	11	3.1	1.6	2.2
40	14	3.2	1.7	2.2	11	2.4	1.1	2.0
41	14	3.0	0.9	2.2	11	2.3	1.6	2.0
42	13	2.6	1.6	2.1	11	2.5	1.6	2.1
43	12	2.7	1.6	2.1	10	2.6	1.4	2.0
44	11	2.4	1.3	2.0	10	2.7	1.4	1.9
45	11	2.6	1.3	2.1	10	3.0	1.5	2.1
46	11	2.8	2.0	2.3	10	3.0	1.5	2.1
47	11	2.8	1.7	2.1	10	2.8	1.4	2.0
48	10	3.1	1.6	2.4	10	2.1	0.8	1.8
49	10	2.7	1.7	2.2	10	2.6	1.5	1.9
50	10	2.6	1.5	2.2	10	2.7	1.5	2.0
51	7	2.7	1.5	2.2	9	2.3	1.1	1.9
52	6	2.7	1.4	2.1	8	2.3	1.5	1.9
53	6	2.6	1.1	2.1	7	2.2	1.1	1.7
54	4	2.5	1.7	2.2	6	2.2	1.3	1.8
55	4	2.2	1.6	1.9	6	2.2	1.3	1.8
56	4	2.6	1.2	2.0	5	2.3	1.7	1.9
57	3	2.3	1.3	1.9	4	2.1	1.8	1.9
58	3	2.4	2.0	2.2	4	2.4	1.5	1.9
59	3	2.3	1.4	2.0	4	2.3	1.4	1.8
60	3	2.1	1.8	2.0	4	2.3	1.6	1.8
61	3	2.3	1.3	1.7	2	2.2	1.1	1.6
62	3	1.9	1.3	1.6	2	2.2	1.8	2.0

for their age. Their weights were, therefore, included in the averages of those for the 9-day suppression period. The averages of all body weights and the average food consumption have been used in plotting the maintenance curves and food eaten per day for each sex in the respective groups. On refeeding it was found that the mice of both sexes fell into one of two groups, namely, those that attained the weight normal for their age before the time selected for the second suppression

TABLE 5

Food consumption per day of normally growing mice at varying body weights. Body weight 7 to 27 grams

BODY WEIGHT	MALES				FEMALES			
	Number of animals	Food eaten			Number of animals	Food eaten		
		Maximum	Minimum	Average		Maximum	Minimum	Average
grams		*grams*	*grams*	*grams*		*grams*	*grams*	*grams*
7–8	1			1.1				
8–9	2			1.2	2			1.0
9–10	6	1.7	1.2	1.5	4	1.6	1.0	1.2
10–11	12	1.8	1.2	1.5	7	1.7	1.0	1.3
11–12	14	2.2	1.3	1.7	8	1.9	1.0	1.5
12–13	14	2.3	1.4	2.0	10	2.3	1.7	2.0
13–14	15	2.3	1.5	2.0	10	2.4	1.8	2.1
14–15	15	2.4	1.6	2.1	11	2.8	1.9	2.2
15–16	15	2.9	1.9	2.3	11	2.6	2.0	2.2
16–17	15	3.0	1.4	2.3	11	2.7	1.8	2.2
17–18	15	3.2	1.9	2.4	11	2.8	1.6	2.2
18–19	14	3.2	1.9	2.4	10	2.7	1.8	2.2
19–20	14	3.0	1.9	2.5	6	2.7	1.9	2.2
20–21	10	3.1	2.0	2.5	2	2.2	2.1	2.2
21–24	4	Aver. for 10 days.....		2.8				
25–27	2	Aver. for 30 days.....		2.4				

period and those that did not. These groups are marked *A* and *B* on the charts. A comparison of the body weights and average daily food consumption of the different groups for the varying periods of suppression of growth is shown in table 6. (In the data submitted in tables 6 and 8, 5.1 Calories per gram have been used in estimating the energy value of the food.) The curves of growth may be seen on charts II to V.

TABLE 6

Body weights and average daily food consumption during varying periods of suppression of growth

NUMBER OF MICE	SUPPRESSION		PERIOD OF SUPPRESSION						FOOD CONSUMPTION				
			Initial body weight			Final body weight			Average daily food intake			Calories estimated	
	Number	Days	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Average daily	Per gram body weight
			grams	*grams*	*grams*	*grams*	*grams*	*grams*	*gram*	*gram*	*gram*		
Males													
17	1st	5	13.4	7.0	9.2	13.4	7.9	9.5	1.2	1.0	1.1	5.6	0.6
11	1st	9	15.5	8.2	10.5	14.0	8.1	10.7	1.5	1.1	1.3	6.6	0.6
8	1st	18	11.8	8.4	10.7	11.8	10.2	11.2	1.4	0.7	1.3	6.6	0.6
6	1st	27	13.4	9.7	11.2	13.5	10.0	11.8	1.5	0.8	1.3	6.6	0.6
Females													
17	1st	5	12.7	6.8	10.2	12.5	8.3	10.1	1.2	1.0	1.1	5.6	0.6
16	1st	9	14.3	6.9	8.8	13.9	8.2	9.8	1.3	1.1	1.2	6.1	0.6
8	1st	18	11.0	8.5	9.8	11.6	9.4	10.3	1.3	0.8	1.2	6.1	0.6
7	1st	27	13.1	9.6	11.0	13.4	10.7	11.7	1.5	0.8	1.2	6.1	0.5
Males													
9	2d	5	18.5	13.7	15.7	18.2	13.7	15.2	1.5	1.4	1.5	7.6	0.5
3	2d	9	17.2	10.3	12.7	16.4	10.3	12.5	1.7	1.2	1.3	6.6	0.5
Females													
15	2d	5	16.1	12.4	13.8	15.8	12.0	13.9	1.5	1.4	1.4	7.1	0.5
8	2d	9	17.1	14.4	15.2	16.2	13.3	14.8	1.5	1.3	1.3	6.6	0.4
Males													
3	3d	5	18.2	13.0	15.3	18.1	13.3	15.1	1.5	1.2	1.4	7.1	0.5
2	3d	9	14.7	11.8	13.2	14.6	12.1	13.3	1.6	1.2	1.3	6.6	0.5
Females													
8	3d	5	17.9	15.7	16.5	17.0	14.1	15.7	1.6	1.6	1.4	7.6	0.5

Reduction in the food requirement as a result of continued underfeeding. A lowered food requirement as the result of continued underfeeding has been reported for cattle by Waters ('11) and by Van Ewing and Wells ('15), and for rats by Jackson ('15). In the present experiment

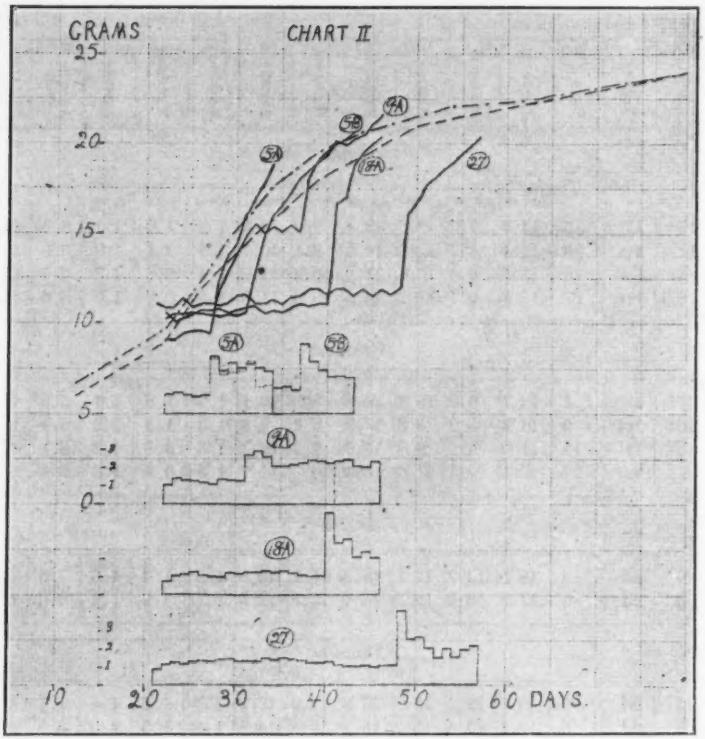

Chart II. Curves showing rapid acceleration of growth after suppression of growth for 5, 9, 18 and 27 days. Males. Normal growth - - - - Judson, -.-.- Thompson, Accelerated growth ——.

there was *in all cases of maintenance a gradual decrease in the amount of food per unit of body weight required to maintain the same weight.* In a few instances, in which the food was unchanged in amount, there is a slight rise in the curve of body weights. In the 18- and 27-day suppressions—extensive periods for such rapidly growing animals

as mice—a slight upward curve was allowed because Jackson ('15) and Stewart ('16) had found it difficult to hold rats to constant weight and keep them alive for a long period.

Changes in the food requirement in repeated periods of underfeeding. In the second and third suppressions the food consumption remained

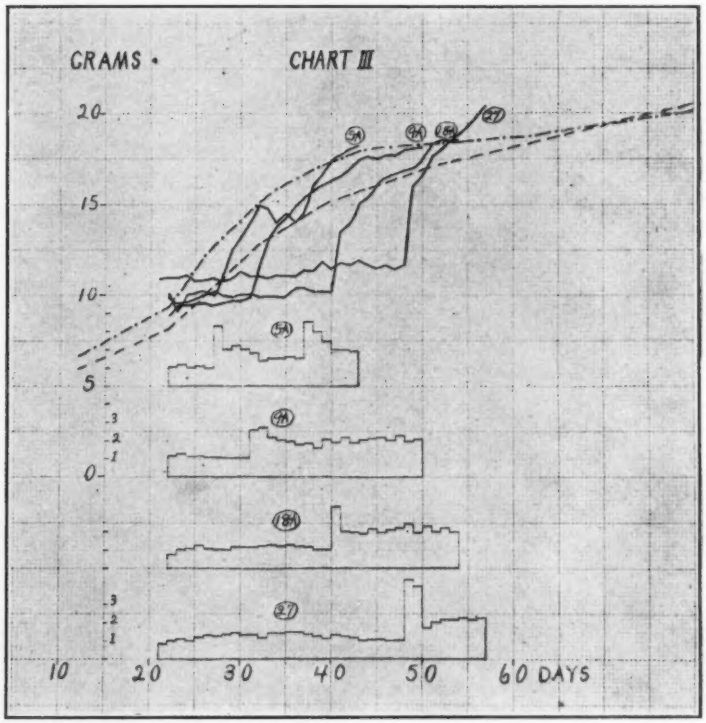

Chart III. Curves showing rapid acceleration of growth after suppression of growth for 5, 9, 18 and 27 days. Females. Normal growth; - - - - Judson, -.-.- Thompson, Accelerated growth ——.

about uniform during the period. There was an increase in actual amount eaten as compared with the first period; but it must be remembered that the mice were at a higher plane of body weight. When food is considered per gram body weight the decrease in food require-

ment in a second and third period is evident (see table 7). These results are in accord with those reported by other investigators. Howe and Hawk ('11) found, after starving the same dog twice for more than 100 days each time, that the total output of nitrogen was 7.1 per cent

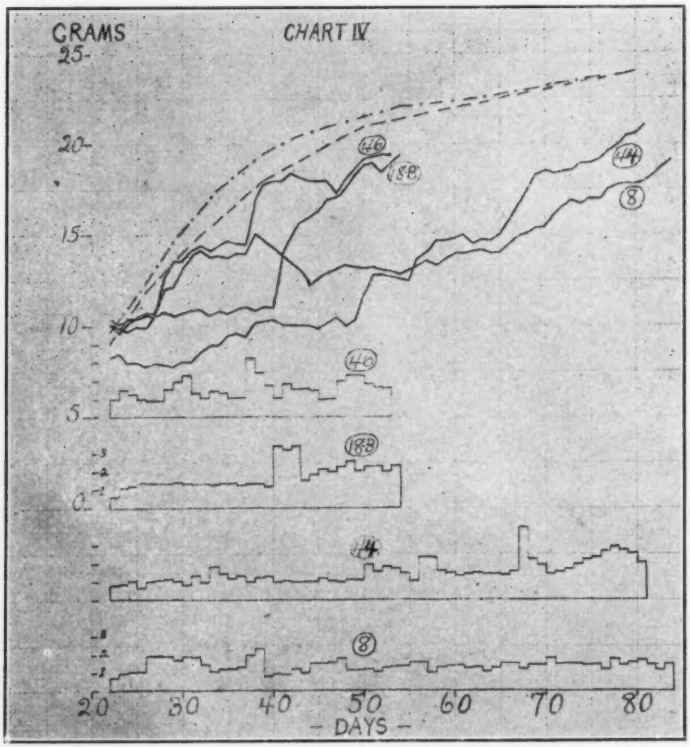

Chart IV. Curves showing moderately accelerated growth after suppression of growth. Males (46), (18-B). Repeated suppressions (44), (46). Slow growth on a small daily food intake (8). Normal growth; - - - - Judson, -.-.- Thompson. Growth after suppression ———.

less in the second fast than in the first. The loss of weight was 10.3 per cent less in the second fast and 21.7 per cent less in the second half of the second fast than in the corresponding periods of the first fast.

Stewart ('16) reported the loss of weight for two rats as slightly less in a second fast than in an initial period of about the same duration.

Comparison of food requirement in suppressed growth with that for normal growth in mice of the same weight. For normal, growing mice the average daily food intake at a size represented by 9 to 10 grams body weight is 1.5 gram for males and 1.2 gram for females. During

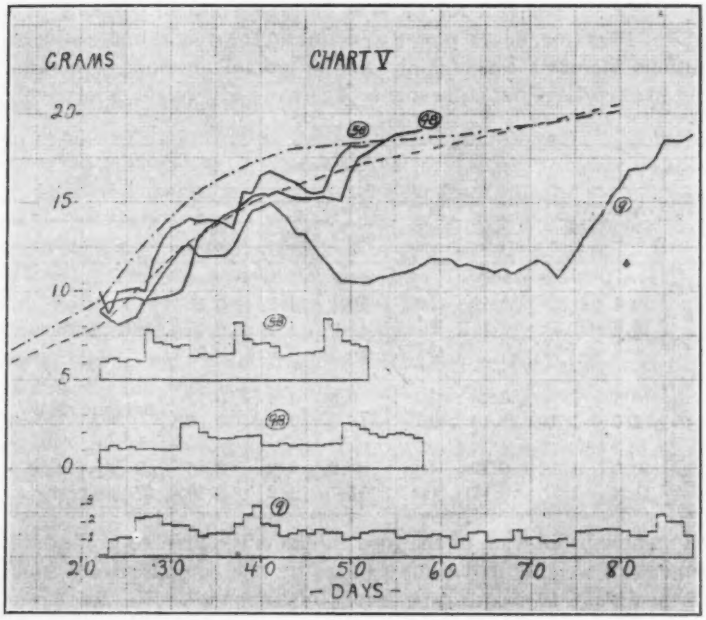

Chart V. Curves showing moderately accelerated growth after suppression of growth. Females. Repeated suppressions (5-B), (9-B). Growth after continued failure to grow (9). Normal Growth - - - - Judson, -.-.-.- Thompson. Growth after suppression ———.

maintenance without growth 1.1 to 1.2 gram is eaten. Growing males weighing 10 to 11 grams need 1.5 gram of food, of the sort here employed, per day for growth; females need 1.3 gram. Mice of the same weight are maintained without growth on 1.2 gram. An 11- to 12-gram male mouse grows normally on 1.7 gram of food; females need 1.3 gram. Thirteen-gram mice require an average food intake

of 2 grams per day for growth, whereas, for maintenance alone, 1.3 gram is sufficient. At 15 grams body weight growing mice eat 2.2 grams per day, while retarded one can maintain constant weight on 1.3 gram. From these differences it may be estimated that about 14 per cent of the food is used for growth at 9 to 11 grams body weight. After the 11-gram stage either the proportion of the food required for growth increases markedly, averaging 19 per cent for mice at 12 grams body weight, 35 per cent for those at 13 grams and 40 per cent at a weight of 15 grams; or the power to maintain the weight under adverse conditions increases considerably as the animal develops. In other words, in mice, large but still capable of gain in body weight, a relatively

TABLE 7

MALES				FEMALES				
Number of suppression	Average body weight	Average daily food intake	Food per gram body weight	Number of suppression	Average body weight	Average daily food intake	Food per gram body weight	
	grams	*gram*	*gram*		*grams*	*gram*	*gram*	
1st	9.4	1.1	0.12	1st	10.1	1.1	0.11	5-day periods
2d	15.0	1.5	0.10	2d	13.9	1.4	0.10	
				3d	15.6	1.0	0.07	
1st	10.6	1.3	0.12	1st	9.8	1.2	0.12	9-day periods
2d	12.6	1.3	0.10	2d	15.4	1.3	0.08	
1st	10.2	1.3	0.12	1st	9.9	1.2	0.12	18-day period
1st	11.8	1.3	0.11	1st	11.7	1.2	0.10	27-day period

much greater proportion of the food intake is required to produce an increment in weight, if one may judge by the comparatively small quota necessary to insure satisfactory maintenance. No account is taken in such generalization, however, of the content of water in the new tissues at the different stages of growth. There is a suggestion also in the relative number of mice responding promptly to refeeding to indicate that the smaller animals may be somewhat less capable of recovering from suppression of growth than are the larger ones.

Some physiological effects of underfeeding. In addition to lowering the food requirement for maintenance and bringing about a better endurance of a second period of restricted diet, underfeeding has been reported as causing changes in general appearance, in body proportions, in rate of growth of various organs, in sex development, in activity and behavior and in intelligence.

Weiske ('75) and Van Ewing and Wells ('14) found increased thickness of hair in cattle. Osborne and Mendel ('11), Wheeler ('13) and Judson ('16) all report changes in the hair coat of rats and mice. Increase in height in proportion to width has been observed in calves by Waters ('00) and by Falke ('10); changes in the relation of height to length were reported for dogs by Aron ('10); increased ratio of tail to body length in rats by Jackson ('15), Stewart ('16) and in mice by Judson ('16). Other features are reported by Eckles, by Jackson and Lowrey ('12), by Jackson ('13–'15), by Aron ('14) and by Stewart ('16).

With respect to bodily activity, the extreme restlessness of underfed rats and mice has been noted by Osborne and Mendel ('11), by Wheeler ('13) and by Judson ('16). Waters and Van Ewing and Wells have observed restlessness in underfed calves. These investigators also reported viciousness and retarded intelligence. In the present experiment restlessness was the most noticeable characteristic which distinguished an underfed mouse from a well nourished one.

During retarded growth the mice were thin-bodied when stretched out, but sat, when quiet, in a hunched position. The body proportions showed the changes mentioned by Judson ('16). The nose was sharp, face narrow, head large and tail length in proportion to the body increased. The fur became rough and matted in the subjects of the earlier experiments. After yeast was added to the food there were few cases of moist looking fur but more often a thicker, drier growth of hair than usual. It was noticed that control mice making very rapid gains had thick fluffed-out fur. Mouse 87 (male) which grew from an initial weight of 10.5 grams at 21 days of age to 20.6 grams at 31 days showed this unusual growth of fur. Mice which grew at about the average rate exhibited the smooth, glossy fur characteristic of normal adults.

The curvatures in the spinal column, due to the fact that underfeeding arrests the growth of the muscles and skin but not that of the skeleton, were easily felt with the fingers. Pronounced single curvatures showed in the pictures of several mice, and a double curvature in that of mouse 95. Mouse 95 (female) increased in weight from 10.9 grams on the 27th day of suppressed growth to 19.5 grams in 9 days of full feeding. At the time the second picture was taken, the curvature had almost disappeared.

To what degree, if any, the effects of underfeeding become permanent is still a matter for investigation. Disproportionate forms have been found in children by Fleischner ('06), but are reported as not occurring

in rats by Osborne and Mendel ('11). According to Waters ('08) height in calves is not altered by underfeeding unless complete retardation is continued for more than 2 or 3 months. After resumption of growth calves approach normal height more nearly than normal width. Aron ('11) thought dogs were permanently stunted in size by underfeeding, the injury being greatest in the youngest animals. He found, however, that rats were not permanently stunted in size when underfed for 150 days though he regarded the development as incomplete. Osborne and Mendel ('11) have found that rats are not necessarily undersized in later growth, even after being stunted for very long periods. They have found also that "suppression at any size does not alter the capacity to grow." By stunting rats in the nursing period Brüning ('14) prevented recovery of normal size for at least 54 days. Stewart ('16) found that in rats there is usually recovery of body weight.

Osborne and Mendel ('15) reported that the "procreative functions are not necessarily impaired by stunting before breeding is ordinarily possible." Two of their rats resumed growth at about 250 days and bore young at 310 days. "There was no damage to the maternal function." No observations were made in the present experiment upon sex development as affected by nutrition. One male and one female after enduring repeated suppressions of growth were used for breeding. The young from these animals seemed normal in every way and showed the usual marked acceleration after a preceding period of suppression of growth.

RESUMPTION OF GROWTH ON ADEQUATE DIETS AFTER RETARDATION OF GROWTH

Characteristics of renewal of growth. The most noticeable characteristics of renewal of growth are the sudden decrease in physical activity; the rapid change in the appearance of the hair coat—the fur becoming smooth and glossy in a few days; the change to normal ratio of tail to body length; and the rapid development of integument and musculature which gives the shape and proportions normal for the body weight. While a few cases of permanent stunting have been observed, the rate of growth, instead of being decreased, proves to be accelerated after suppression. This has been observed in the cat by Schapiro ('05), in the rat by Hatai ('07), in the salamander by Springer ('09) and by Morgulis ('11), in the child by Schloss ('11), by Boas ('12) and by Hess ('15), in the rat by Ferry ('13) and by Osborne and Mendel ('15 and

'16), and by others. That the rate of growth does not decrease with the age of the animal has been shown by the resumption of growth in rats fed by Osborne and Mendel at "more than twice the age at which adequate size is ordinarily reached, the growth after suppression being comparable in most cases to that of a growing rat of the same size and sex." Seland had observed as early as 1888 that rabbits and chickens enduring alternate short periods of fasting and liberal feeding grew to a weight heavier than the controls. Noè ('00) found little or no overcompensation in body weights in rats refed after repeated periods of starvation. In the salamander, in which there is probably a large absorption of water, it was found by Morgulis ('11) that the increase in body weight after refeeding may be even greater than the weight of the ingested food. Brüning ('14) failed to cause "compensatory overgrowth in rats placed on an artificial diet after being subjected, during the suckling age, to repeated periods of fasting." In investigations by Waters the food consumption and rate of gain in cattle refed after being kept on maintenance from 6 to 12 months, were about twice that of the control animals. Stewart ('16) reported that after short periods of maintenance (40 to 42 days) rats were able to overtake but not to exceed the weight of the controls.

In the present experiment most of the curves of growth resulting from full feeding after suppression of growth show noticeable acceleration. Practically all of the animals exceeded the normal curves given by Judson and most of them reached or exceeded in weight the controls grown in this laboratory. This was not true of the males suppressed 27 days although they made an average absolute gain equal to that made by the females during the 9 days of refeeding. The mice for the 27-day suppression were selected from three litters and were more alike than any other test mice. They were all 21 days old at the time the experiment began. The females were considerably larger for their sex than were the males. The fact that the females exceeded their controls in growth while the males did not might indicate, as was suggested before, that the larger the animal the less the injury from suppression. It should be mentioned that all of these mice were bred from male 8 which had endured three 5-day suppressions and finally reached a weight of 20 grams after 67 days in an experimental cage. In the 27-day group all individuals grew at rapid rates during the 9 days of refeeding. The extremes in percentage gain were 91 per cent made by mouse 96 (male) and 56 per cent made by mouse 84 (female). The heaviest weight was that of mouse 82 (female), 23.1 grams, the lowest was mouse 89 (female), 18.6 grams.

In all other periods of suppression there were at least two groups with regard to the rate of growth after refeeding. This division formed the basis of selection for the second suppression. The mice gaining most rapidly were continued on a liberal diet while the others were again put upon limited food. After a 5-day suppression the males gained a little more rapidly than did the females. However all but three were suppressed in growth again. All of the females were suppressed a second time even though many of them had overtaken their controls at the close of the first refeeding. Both males and females regained the weight normal for their ages in spite of a second or even a third suppression. In other words, even after repeated suppression of growth the growth impulse provokes a vigorous response to suitable diet, as shown by the decided acceleration of gain in weight. This corresponds with what has been observed in rats. There was a greater variation among the males with regard to the rate of resumed growth than was displayed among the females.

The 9-day group contains practically all of the earlier experiments before yeast was added to the diet. All but two males overtook their controls in 9 days of full feeding; one, 17, did not respond to a second suppression, and another, 44, started as a control and, after it had limited its own growth by inadequate eating for 18 days, it was subjected to two rather severe restrictions of diet with alternate periods of refeeding. This treatment seemed to bring about growth as well as regular habits of eating. This mouse did not, however, develop into a well proportioned adult. The abdomen was large and the body short and thick.

The mice suppressed in growth 18 days all recovered, but at two general rates of growth as in the 5- and 9-day groups. Six males made as high absolute gains in six days as the mice in which growth had been suppressed 27 days did in 9 days. The curve that flattened most was in the groups of females and represents only two individuals.

Lengthening the period of suppression might be expected to result in a more uniform rate of recovery. This is not, however, the only explanation of such a result. The males in the 18-day group and all of the mice in the 27-day groups were more uniform in weight and larger than the majority of the animals in the other tests. The curves of these three groups show the most uniformly rapid growth. There are individual exceptions to this rule, as is the case with two of the males recovering rapidly after 5 days of retardation. These two mice, 74 and 77, weighed 8.2 and 7.9 grams respectively when the test began.

It was difficult to keep them from growing. After refeeding 6 days, their weights showed an increase of 120 per cent and 125 per cent respectively. Only one other experimental mouse made as high a gain per cent and that one, 33, was larger at the beginning of limited feeding.

Statistics of daily food consumption during realimentation after periods of suppressed growth for 5, 9, 18 and 27 days. The groups of male and female mice in the different suppression tests have been arranged in table 8 according to their rate of growth during refeeding. The comparative food intake, as would be expected, runs parallel with the growth. Mice growing at greatly accelerated rate consume an average of from 2.1 to 2.9 grams per day which is more than the average for controls of the same age. Control mice weighing the same as the test mice at the beginning of refeeding consume from 1.5 go 1.7 gram per day. The maximum food eaten by some of the controls during their growth from 12 to 20 grams body weight (26 to 41 days) exceeded the average maximum per day of any animal refed after suppression of growth. The highest food consumption among the test animals was 3.1 grams per day by males in the group suppressed in growth 18 days and by females in the 27-day suppression test. The average growths in these cases were greatly in excess of the average for the controls (charts II, III). The average food consumption was approximately the same as that of the most rapidly growing controls of the same age. The growth was considerably in excess and the food intake somewhat greater than that of any controls of the same size. Mice growing at moderately accelerated rate after suppression consumed approximately the same amount as controls of their age.

A few mice proved to be capable of long continued growth and finally reached adult size on a daily food consumption only slightly above maintenance. Mice 8 and 44 (males) and 9 (female) are reported in table 8 as examples of such growth capacity. Mouse 8 reached a weight of 19.6 grams at the age of 63 days after three 5-day suppressions of growth. It ate, during the entire period of refeeding, an average of but 1.5 gram of food per day. Two grams is the average daily food intake of mice weighing 11 to 12 grams at about 26 days of age. Mouse 44 after two 9-day suppressions increased its body weight from 8.1 grams at 32 days to 20.3 grams at 62 days on an average of 1.6 gram of food per day. This mouse had maintained its weight in the first suppression on 0.9 gram of food per day and in the second period of restricted diet on 1 gram per day showing an unusually low food requirement. After the third 5-day suppression, mouse 9 was continued on a full diet

TABLE 8

Gains in weight and average daily food consumption during periods of refeeding after varying periods of suppression of growth

CHART NUMBER	NUMBER OF MICE	PREVIOUS SUPPRESSION DAYS	REFEEDING DAYS	GAINS IN BODY WEIGHT				FOOD CONSUMPTION				
				Absolute gain			Average gain per cent	Average total food intake	Average daily food intake			Calories estimated average daily intake
				Maximum	Minimum	Average			Maximum	Minimum	Average	
Accelerated growth. Males												
				grams	*grams*	*grams*		*grams*	*grams*	*grams*	*grams*	
II, 5-A	3	5	7	10.8	7.4	9.5	105	19.2	2.8	2.7	2.7	13.8
II, 18-A	3	18	6	9.5	7.1	8.3	72	17.3	3.1	2.5	2.9	14.8
II, 5-B	6	2 x 5	11	12.9	8.8	10.6	108	27.5	2.9	2.2	2.5	12.8
II, 9-A	7	9	9–10	11.8	6.5	9.1	83	22.7	2.7	2.1	2.4	12.2
II, 27	5	27	9	9.6	6.2	8.3	70	21.0	2.5	2.2	2.3	11.7
Accelerated growth. Females												
III, 27.....	5	27	9	9.7	7.4	8.5	73	24.8	3.1	2.5	2.8	14.3
III, 5-A...	5	2 x 5	11	9.3	7.3	8.3	81	25.6	2.6	2.1	2.2	11.2
III, 9-A...	6	9	13	9.2	6.3	7.8	82	26.7	2.2	2.0	2.1	10.7
III, 18-A...	6	18	14	8.6	5.8	7.9	74	31.4	2.3	2.2	2.2	11.2
Moderately accelerated growth. Males												
IV, 18-B...	4	18	11–14	10.4	7.1	9.0	84	31.3	2.5	2.1	2.4	12.2
IV, 46.....	1	3 x 5	15	8.6			80	30.1			2.0	10.2
Moderately accelerated growth. Females												
V, 5-B...	8	3 x 5	15–16	10.7	5.9	8.6	88	36.8	2.6	2.0	2.4	12.2
V, 9-B...	6	2 x 9	18	11.5	6.2	8.9	87	37.2	2.3	1.8	2.1	10.7
Slow growth. Males												
IV, 44.....	1	3 x 9	31	7.6			73	51.0			1.6	8.2
IV, 8.....	1	3 x 5	55	9.5			89	82.4			1.5	7.7
Slow growth. Females												
V, 9.....	1	3 x 5	57	9.2			98	75.7			1.3	6.6

47 days. During this 47-day period it grew to the average weight of females 62 days of age on an average daily food consumption of 1.3 gram.

Comparison of the average total food consumption during realimentation with food eaten by controls in the time required to make the same growth from the same initial weight. The total food required for males to make such absolute gains as are shown in the different periods of refeeding is compared in table 9 with the food eaten by control males growing with-

TABLE 9

Comparison of the average total food consumption of male mice during refeeding after varying periods of suppression of growth with food eaten by controls in the time required to make the same gain from the same initial weight

FOOD AND GROWTH CURVES CHART NUMBER	NUMBER OF MICE	GAIN IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN			GAIN IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIOD IN WHICH THE GAIN ACTUALLY OCCURRED
				Suppression period	Refeeding period	Average total	
		grams		*grams*	*grams*	*grams*	*per cent*
II, 18-A	3	8.3	6	24.8	17.3	42.1	48
II, 27	5	8.3	9	34.2	21.0	55.2	40
(Controls	15	8.3	16			31.2	26)
II, 9-A	6	9.1	9–10	11.8	22.7	34.5	40
IV, 18-B	4	9.0	12	21.6	28.3	49.9	32
(Controls	15	9.0	17			34.9	26)
II, 5-A	3	9.5	7	5.8	19.2	25.0	50
(Controls	15	9.5	16			31.6	29)
II, 5-B	6	10.7	11	12.9	27.5	40.4	39
(Controls	15	10.7	21			40.5	26)

In making these comparisons, no account has been taken of the content of water in the new tissues.

out retardation in the time necessary for them to make the same gain in body weight from corresponding initial weights. The food consumption of the controls has been estimated from table 4. The averages for food consumption during the various periods of suppression were taken from the daily records of each group. The figures differ from those

given in table 6 because of the exclusion in this instance of the animals killed for measurement at the end of the various suppression periods. Data similar to those given for the males are presented for the females in table 10.

TABLE 10

Comparison of the average total food consumption of female mice during refeeding after varying periods of suppression of growth with food eaten by controls in the time required to make the same gain from the same initial weight

FOOD AND GROWTH CURVES CHART NUMBER	NUMBER OF MICE	GAIN IN BODY WEIGHT	DAYS REQUIRED FOR GAIN	FOOD EATEN			GAIN IN BODY WEIGHT COMPARED WITH FOOD CONSUMED DURING THE PERIOD IN WHICH THE GAIN ACTUALLY OCCURRED
				Suppression period	Refeeding period	Average total	
		grams		*grams*	*grams*	*grams*	*per cent*
III, 9-A	6	7.8	13	11.1	26.7	37.8	29
III, 18-A	6	7.8	14	21.6	31.4	53.0	25
(Controls	11	7.8	25			51.0	15)
III, 27	5	8.5	9	34.1	24.8	58.9	34
(Controls	11	8.5	29			54.5	16)
III, 5-A	5	8.5	11	13.0	25.6	38.6	33
V, 5-B	8	8.6	15–16	20.1	36.6	56.8	24
(Controls	11	8.5	24			50.2	16)
V, 9-B	6	8.9	18	22.6	37.2	59.8	24
(Controls	11	8.9	31			57.4	15)

In making these comparisons no account has been taken of the content of water in the new tissues.

Three males which had been suppressed in growth 5 days lived through the maintenance period and made rapid growth on less total food than the controls ate, while their weight increased by the same absolute amount. These mice, group A-5, males, include the two very rapidly growing individuals, mentioned above, which overtook their controls in 4 days. The average food eaten by the members of this group was 25 grams. Of this amount 19.2 grams were eaten during the period of accelerated growth. The gain in body weight corresponded to 50 per cent of this intake. In making the same gain, control mice ate 31.6

grams of food and retained an equivalent of 29 per cent as body weight. If mice 74 and 77 are considered without the other member of this group, it is found that their average food consumption for the period of refeeding (6 days) was 19.4 grams. During this time their average gain was 10.6 grams. Approximately 55 per cent of the weight of the food was added to the weight of the body. In calculating the gains of weight in terms of food intake, no account has been taken of the water factor. Obviously a considerable fraction of the weight put on represents water in the new tissues. Stewart reported that an average of 16 per cent of the ingested food (exclusive of water) was applied toward the increment in body weight of his test rats.

The mice suppressed in growth 9 days and group B in the 5-day test ate approximately the same amount of food as did their controls. These groups also show high gains in body weight in comparison with the total food consumption in the period of accelerated growth. *In all cases the weight gained by the experimental, i.e., stunted, animals was greater in proportion to the food eaten than that gained by the controls.*

Among the females recovering rapidly from suppression of growth, groups 5-A and 9-A show a utilization of smaller amounts of food than was eaten by the controls in making the same gain in weight. All other groups show a greater cost of growth, in terms of total food consumption, when the food intake during the period of underfeeding is also taken into account. From the results of this study it appears that, in normally growing white mice, increase in body weight, in the twenty days following weaning, is equivalent to from 20 per cent to 30 per cent of the total food eaten. In mice growing at accelerated rate after suppression of growth, the gain may be as high as 50 per cent to 55 per cent of the food eaten during the period of actual growth. The added expense of food for maintenance during the period of suppression of growth makes the *total economy* of food unfavorable when long periods are involved.

SUMMARY

From new statistics of the body weight determined daily for mice, curves of normal growth have been prepared. These indicate that up to the 26th day of life the actual gain per day is approximately the same for both sexes. After the 26th day the males continue to grow with comparative rapidity until about the 40th day, whereupon the slower, gradually diminishing rate of increment ensues. The females gain from the 26th day at the rate of 0.5 gram per day until the 34th day, when their curve flattens perceptibly.

The normal daily food consumption has been ascertained on a large scale during the period of growth between the 22d and the 62d day of life. The diet consisting of a mixture of comparatively simple food products, was selected to insure adequate proportions of all essential nutrients.

During prolonged periods of suppressed growth with a stationary body weight, the food requirement, as measured by the actual ad libitum intake, decreases after a time and remains at an apparent minimum level throughout the period of underfeeding. In second and third suppressions of growth after intervening periods of accelerated growth, the food requirement, in proportion to the body weight, is less than in the first period of retarded growth.

In correspondence with the observations of previous investigators the present experiments have shown that the resumption of growth after the suppression of growth, during periods of varying lengths at different stages of the growth cycle, ensues at a greatly accelerated rate.

The daily food intake has been determined during the period of rapid or accelerated growth which followed suppression of growth under a variety of experimental conditions. In this way it has become possible to compare the increment of body weight in relation to the food intake under widely varying physiological conditions of growth for comparable increments in individuals of the same size.

Comparisons of the economy of the food intake show that the gain of weight during the period of acceleration of growth following suppression of growth is ordinarily accomplished on a smaller intake of food than is ingested during a period of equal growth at normal rate from the same initial body weight. The advantage in this apparently better appropriation of food during accelerated growth may actually be sufficient, in some cases, to offset the added expense of the food required for maintenance without growth during a brief preliminary period of suppression. In other words, in several instances, the total quantity of food ingested during the entire period included in the failure to grow and the restitution of growth up to the normal size taken for comparison, has been no greater than that consumed by unstunted animals making the same gain of body weight at the normal rate. Ordinarily, however, the food requirement during any considerable preliminary period of maintenance without growth is sufficient to overbalance any economy in food during the period of accelerated growth.

Slow but completed growth may be accomplished even with a very small daily food intake.

Problems relating to changes in form incident to the suppression of growth are discussed. Whether permanent changes in proportions of stature actually remain after the compensatory growth following early stunting needs to be investigated more extensively.

The probable influence of the size of the animal, expressed in body weight, upon the rate of resumption of growth has been noted.

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DESCRIPTION OF FIGURES

No. 96a. Male. Initial weight, 9.7 grams. Picture taken 27th day of suppression of growth. Weight, 10 grams. Age, 47 days.

No. 96b. Male. 9th day of refeeding. Weight, 19.1 grams. A gain of 91 per cent in 9 days. Age, 56 days.

No. 95a. Female. Initial weight, 10.4 grams. Picture taken 27th day of suppression of growth. Weight, 10.9 grams. Age, 47 days.

No. 95b. Female. 9th day of refeeding. Weight, 19.5 grams. A gain of 79 per cent in 9 days. Age, 56 days.

No. 99a. Male. Initial weight, 11.8 grams. Picture taken 18th day of suppression of growth. Weight, 11.8 grams. Age, 41 days.

No. 99b. Male. 6th day of refeeding. Weight, 20.2 grams. A gain of 71 per cent in 6 days. Age, 47 days.

No. 104a. Female. Initial weight, 10.4 grams. Picture taken 18th day of suppression of growth. Weight, 10.7 grams. Age, 41 days.

No. 104b. Female. 13th day of refeeding. Weight, 19.3 grams. A gain of 80 per cent in 13 days. Age, 54 days. This mouse had reached a weight of 19 grams on the 10th day of refeeding.

No. 31. Control male. Weight, 13.0 grams. Average weight and normal form of males, 22 to 25 days of age.

No. 116. Control male. Weight, 21.4 grams. Age, 50 days. Average weight and normal form of adult males. This mouse grew at the exact rate of the average curve, chart 1.

Fig. 1 96 (a)

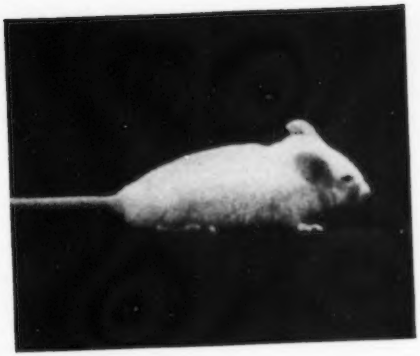

Fig. 2 96 (b)

Fig. 3 95 (a)

Fig. 4 95 (b)

Fig. 5 99 (a)

Fig. 6 99 (b)

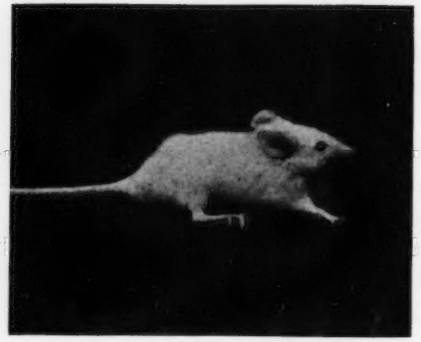

Fig. 7 104 (a)

Fig. 8 104 (b)

Fig. 9 31

Fig. 10 116

OROKINASE AND PTYALIN IN THE SALIVA OF THE HORSE

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The literature upon this subject has been rather thoroughly and painstakingly reviewed in three papers of recent publication. Palmer, Anderson, Peterson and Malcomson (1), R. J. Seymour (2) and Hayden (3) have reported upon different studies concerning salivary digestion in the horse. It is the purpose of this paper to submit data gathered more recently upon the subject and to compare the results with those reported in the preceding work. Palmer (1) and his coworkers report the existence of an enzyme, present in the buccal and possibly the lingual glands, that activates a ptyalinogen present in the secretion of the salivary glands of the horse. They conclude from the evidence of their data that neither saliva obtained from a parotid fistula nor a glycerine extract of the parotid gland digests starch. They also show that the mixed mouth secretions obtained from an esophageal fistula are more powerful in their amylolytic action than those obtained from the mouth. The name orokinase is given to the activating enzyme which activates the parotid saliva and the claim is made by them that the enzyme is present in the mouth secretions of both horse and man.

In Seymour's work mixed saliva of the horse is shown to have produced reducing sugar after eight hours digestion. The parotid saliva does the same thing with slightly accelerated action. Old and recent glycerine extracts of the salivary glands of the horse are negative up to eighteen hours. Water extracts were positive after six hours digestion with a 2 per cent corn starch solution. There is no evidence of an enzyme in the mouth secretions that activates a ptyalinogen present in the secretions of the salivary glands. The saliva of the horse, both the mixed and the isolated secretions of the parotid and submaxillary glands, contains a diastase capable of converting starch into sugar.

The diastase is extremely feeble, requiring at least five hours digestion for the conversion of starch to reducing sugar.

Hayden (3) after having digested a 1 per cent solution of soluble starch with parotid fistula saliva up to twenty-four hours obtained evidence of the presence of an enzyme capable of carrying the digestion of the 1 per cent starch solution to the maltose stage at least. The test for sugar was made with Benedict's quantitative solution.

Carlson, Greer and Becht (4) collected mixed saliva from two horses using pilocarpine as a sialogogue. They report no amylolytic action on the part of those samples. Twelve other samples of mixed horse saliva collected by them showed no power to digest starch.

Carlson and Crittendon (5) inserted a cannula into Stenson's duct. The parotid saliva collected by that method digested starch. The saliva was collected from a human subject.

M. Foster (6) in discussing the action of the saliva of man says that the parotid saliva alone digests starch and that the secretion of the submaxillary gland is sometimes more powerful than that of the parotid.

Prof. E. H. Starling (7) reports the stimulation of the flow of saliva in the dog by the use of pilocarpine with no amylolytic action on the part of the saliva.

Wiley (8) in giving an analysis of the grains says that there is in corn 71.48 per cent of starch, sugar and dextrin combined. Maize flour contains 78.36 per cent of starch and sugar. Unhulled oats contain of starch and sugar 57.93 per cent. Hulled oats contain 67.09 per cent of these substances.

Another author (9) claims that there is practically no dextrin or maltose in untreated grains.

Bradley and Kellersberger (10) report the presence of an active diastase in the seed of both mature and young corn. Jordan (11) says that sugars are formed in small quantities in the hays and in scarcely appreciable quantities in the grains. Saccharose exists in considerable proportion in field corn. Maltose exists in no quantities. Dextrose is found in maize.

Effront-Prescott (12) state that corn, rye and all other cereals contain considerable quantities of amylase and other substances that accelerate diastatic action. The action of the diastase begins during the process of milling. There is a transformation of the starch of the grains into sugar and the action of the amylase is shown at grinding. The amylolytic action takes place more readily in the presence of water.

METHODS AND RESULTS

Parotid saliva from the horse was collected through a fistula of Stenson's duct. Mixed saliva was collected both from the mouth and an esophageal fistula. The parotid saliva was collected while the animal was manger or trough feeding. The flow of mixed saliva was stimulated in various ways. Sometimes it was collected easily, other times with difficulty. Our work thus undergoes the same limitations as that of Palmer et al. (1).

Mixed human saliva was collected from a large number of persons. Parowax was chewed in each case to stimulate the flow. When this saliva was diluted the dilution was 1-50 and two drops of the dilution were used for digestive work.

The various extracts were made with 50 per cent glycerin in water. The glands and mucous membranes were extracted on the day the

TABLE 1

	STARCH	N/20 I	SUGAR
			grams
Pilocarpine crystals	0	G	0.000
Pilocarpine crystals			0.000
Pilocarpine, hypodermic	0	G	0.016
Pilocarpine, hypodermic			0.023+
Arecoline, hypodermic	0	G	0.023+
Arecoline, hypodermic			0.023+
Pilocarpine crystals plus 1 cc. parotid saliva	0	G	0.000

animal was killed and digestion was carried out the next. We have a series of four horses from which extracts have been made.

Unless exception is noted all samples were digested with 5 cc. of a 1 per cent corn starch solution for a period of two hours. Digestion was carried on in an electric incubator at a temperature of 38 to 40°.

The extent of digestion has been indicated by the physical appearance of the starch, the reaction the starch to N/20 I and the quantity of sugar produced. Most of the samples showed considerable starch at the end of the digestion period and unless the clearing was marked we have indicated the result by O. With N/20 I the achroödextrin stage has been indicated by −, the erythrodextrin by R and varying degrees of reaction by G for good, F for fair, S for slight, V. S. for very slight. Benedict's test was used to determine the quantity of sugar produced.

Palmer (13) states that pilocarpine digests starch. We used the

drug in both the crystalline form and in hypodermic tablets in previous work. In the present work we have used hypodermic tablets of arecoline in addition to the two forms of pilocarpine. Table 1 indicates typical results obtained in the use of these drugs.

One-half grain of the respective form of the drugs was dissolved in 10 cc. of water and 2 cc. of the solution used. Several samples of each drug have been tested. There is no evidence of digestive action on the part of either of them. However a considerable amount of reducing sugar is present in the hypodermic tablets of each drug.

Many samples of human saliva diluted 1-50 show a decided digestive power. One sample diluted 1-100 gave good results. Data involving the use of diluted human saliva are indicated in table 2.

TABLE 2

	STARCH	N/20 I	SUGAR
			grams
A	0	R-G	0.002–032
B	0	R-G	0.000–023
C	0	R-G	0.003–032

A contains 2 drops of human saliva in a dilution 1–50.
B is A plus 1 cc. mixed mouth saliva of the horse.
C is A plus 1 cc. parotid fistula saliva of the horse.

Table 2 is a summary of a very large number of samples. The starch solution was not cleared in any of them. The erythrodextrin reaction predominates in A. In B and C the reaction R–G is about even. The average amount of sugar found in A is equal to that of either B or C. From our interpretation of these results we can see no evidence that human saliva diluted 1-50 activates either mixed mouth saliva or parotid fistula saliva from the horse.

TABLE 3

	STARCH	N/20 I	SUGAR
			grams
A	0	– to V.S.	0.017–032
B	0	F-G	0.000–009
C	0	R-G	0.000–001

A is 1 cc. of human saliva.
B is 1 cc. of mixed mouth saliva from the horse.
C is 1 cc. of parotid fistula saliva from the horse.

The physical appearance of the starch was not a good index of digestion in this series. The human saliva changed the starch to achroödextrin in the greater number of the tests. The reaction of either B or C to N/20 I is not to be compared to that of A. The amount of sugar produced in B or C is a negligible quantity when compared with the amount indicated in A. The action of human saliva on cooked starch is much greater than that of mixed or parotid fistula saliva from the horse.

TABLE 4

	STARCH	N/20 I	SUGAR
			grams
A	0	R-G	0.000–011
B	0	R-G	0.000–007

A is 2 drops of mixed mouth saliva from the horse diluted 1–10.
B is A plus 1 cc. parotid fistula saliva.

The erythrodextrin reaction occurs but once in each series. As in the other tables a large number of samples is represented in the summary given in this table. The maximum amount of sugar in either A or B is far above the average of either. The average of each is too nearly equal to be taken as evidence of the presence of an activator in the mixed mouth saliva of the horse.

Repeated tests with corn or oats ground in a food chopper, mixed with water and filtered through cheese cloth give evidence of reducing sugar. The quantity of sugar varies from 0.000 gram in a sample of cornmeal to 0.15 gram in a sample of ground oats. The amount of sugar obtained from either of these grains after having been digested with either mixed or parotid fistula saliva from the horse does not average higher than the grains alone when mixed with water. There is evidence in the literature already quoted of sugar in these grains and of the presence of a diastase that may be activated by grinding them. The diastase is also said to be active in the grains without any mechanical change in them.

Glycerine extracts of the mucosa of the mouth, of the buccal glands, and of the salivary glands from four different horses have not shown any marked activating influence when digested with parotid fistula saliva or in different combinations with each other. We have too few of this series to come to a definite conclusion. Some of the digested products if tested with Fehling's solution alone would show a marked

reduction. Orokinase cannot be demonstrated by the use of that reagent alone. We have a large number of samples in the different phases of the work that would give some considerable reduction. When the quantity of sugar was measured it was found that it was not appreciably greater in the material in which no enzyme was supposed to have been present. Two drops of human saliva diluted 1-50 gave frequent erythrodextrin reactions when digestion was carried two

TABLE 5

Showing the amount of reducing sugar in ground corn and oats. Time of digestion, 2 hours. Sugar reported is the quantity in the whole filtrate

GRAIN	AMOUNT	AMOUNT H_2O	SUGAR
	grams	*cc.*	*grams*
Corn	20	100	0.04
Oats	20	100	0.06
Corn	10	50	0.08
Cornmeal	2	25	0.02
Oats	2	25	0.04
Cornmeal	2	25	0.000
Cornmeal	2	25	0.025
Oats	2	25	0.025
Corn	2	25	0.086
Oats	5	25	0.085
*Corn	5	25	0.019
*Oats	5	25	0.012
*Corn	5	25	0.015
*Oats	5	25	0.017
Oats	5	25	0.15
Corn	5	25	0.10
*Oats	5	25	0.10
*Corn	5	25	0.09
Oats	5	25	0.029
Cornmeal	5	25	0.019

* Indicates no digestion, samples were tested right after mixing with water.

hours. None of the samples tabulated in table 7 did save one and 10 cc. of parotid fistula saliva was used in that. No sugar could be measured from this sample. Parotid saliva in quantities greater than 1 cc. when digestion is carried up to twenty-four hours frequently give the erythrodextrin reaction and measurable quantities of sugar. The same thing has occurred with the mixed saliva and the various extracts that have been made. This seems to be in accord with the results obtained by Seymour (2).

The amount of sugar measured in these cases has been a disappointment to us. We feel that an enzyme in the mouth secretions activating the salivary secretions would lead to the production of measurably more sugar than that produced from the secretions or extracts used alone. That has not been the case in our experiments.

Our one esophageal fistula has not given the results hoped for. The amount of sugar produced in any experiment with the mixed saliva

TABLE 6

Action of saliva of the horse on ground grains. Time of digestion, 2 hours. Five grams of grain to 25 cc. of water in each sample

GRAIN	SALIVA	AMOUNT	SUGAR
		cc.	*grams*
Corn	Mixed	5	0.055
Corn	Parotid	1	0.017
Oats	Parotid	2	0.035
Cornmeal	Mixed	1	0.050
Cornmeal	Parotid	1	0.032
Corn	Mixed	1	0.067
Corn	Mixed	1	0.035
Oats	Mixed	1	0.050
Corn	Mixed	1	0.043
Oats	Mixed	1	0.057
Corn	Mixed	1	0.027
Corn	Mixed	1	0.045
Corn	Mixed	1	0.033
Oats	Mixed	1	0.046
Oats	Mixed	1	0.045
Cornmeal	Mixed	1	0.075
Cornmeal	Mixed	1	0.090
Cornmeal	Parotid	10	0.058

from that source has been small. In no wise has it been comparable with the action of human saliva on either cooked or raw starch.

Neither oats nor corn having been thoroughly masticated and passed through the fistula have given more sugar than we have obtained from the ground grains in water. Mixed saliva from this fistula did not show digestive action on these grains. The mixed saliva did not show any reducing power. Mixed saliva acting on cooked starch for twenty-four hours produced 0.008 gram of sugar. The human saliva in the dilution used produced as much or more when digestion was carried only two hours.

TABLE 7

Action of various gland extracts on starch; 0.5 cc. of extract used. Where parotid saliva is used 1 cc. is the amount. Time of digestion, 24 hours

MATERIAL	STARCH	N/20 I	SUGAR
			grams
Buccal gland	0	G	0.000
Buccal gland plus parotid saliva	0	G	0.000
Parotid saliva	0	G	0.000
Parotid saliva, 10 cc	0	R	0.000
*Buccal gland			0.000
Buccal gland plus parotid extract	0	G	0.004
Buccal gland plus submaxillary extract	0	G	0.002
Buccal gland plus sublingual extract	0	G	0.000
Lingual gland	0	G	0.000
Lingual glands plus parotid saliva	0	G	0.004
*Lingual glands			0.000
Lingual glands plus parotid extract	0	G	0.001
Lingual glands plus submaxillary extract	0	F	0.001
Lingual glands plus sublingual extract	0	G	0.002
Mucosa, mouth	0	G	0.001
Mucosa plus parotid saliva	0	G	0.002
*Mucosa			0.000
Mucosa plus parotid extract	0	G	0.000
Mucosa plus submaxillary extract	0	G	0.001
Mucosa plus sublingual extract	0	G	0.001
Parotid extract	0	G	0.002
Submaxillary extract	0	G	0.001

* Indicates no digestion.

TABLE 8

Material from esophageal fistula

	STARCH	N/20 I	SUGAR
			grams
1. Starch plus mixed saliva	0	G	0.004
2. Starch plus 0.5 cc. mixed saliva	0	S	.003
3. Oats from fistula, 1 cc. of filtrate			0.004
4. Oats from fistula, 1 cc. of filtrate 24 hours			0.003
5. Oats from fistula, 2 cc. of filtrates 0.2 HCl			0.002
6. Starch plus mixed saliva, 24 hours digestion	0	S	0.008
7. Ground corn 2 grams, 25 cc. H_2O plus 1 cc. mixed saliva			0.000
8. Oats ground 2 grams 25 cc. H_2O plus 1 cc. mixed saliva			0.001
9. 1 cc. mixed saliva, not digested			0.000

CONCLUSIONS

1. Pilocarpine hydrochloride does not digest starch. Hypodermic tablets of both pilocarpine and arecoline contain a reducing substance in comparatively large quantities but do not in themselves digest starch.

2. Two drops of human saliva diluted 1-50 carry 5 cc. of a 1 per cent starch solution to the erythrodextrin stage in a large number of cases. A measurable amount of sugar is produced as a result of that digestion.

3. Human saliva in such a dilution does not activate either mixed or parotid fistula saliva from the horse.

4. Mixed human saliva digests cooked starch much more readily than either mixed or parotid fistula saliva of the horse.

5. Two drops of mixed mouth saliva from the horse diluted 1-10 does not activate parotid fistula saliva from that animal. It does not show any appreciable digestive power when used alone in that dilution.

6. The filtrate from a solution of ground corn or oats contains a reducing sugar. The quantity of sugar does not show an average increase when the grains are digested with either mixed or parotid fistula saliva from the horse. Mixed human saliva does digest them under the same conditions.

7. Extracts from the glands and mucosa of the mouth have failed to activate parotid saliva or extracts of the salivary glands of four different horses.

8. Corn and oats passed through an esophageal fistula show no more reducing sugar than the ground grains themselves. Mixed saliva from the esophagus has not shown any marked potency.

9. The glands of the mouth as well as the salivary glands produced a small amount of enzyme that will digest starch within a twenty-four hour period.

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THE INFLUENCE OF PITUITARY EXTRACTS ON THE DAILY OUTPUT OF URINE

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INTRODUCTORY

Since Magnus and Schäfer (1) first pointed out a possible relation between the hypophysis cerebri and renal function, a large amount of work has been done in an attempt to fix this relationship and, if possible, to point out its nature. The results obtained have been quite conflicting. The earlier investigation seemed to show that extracts of the pituitary gland when injected intravenously have a pronounced diuretic effect. The later work would indicate that such extracts give results which are exactly contrary to those obtained by the earlier investigators, namely, an antidiuretic effect.

In this investigation it was our purpose to find out first, whether the subcutaneous injection of pituitary extract will cause any quantitative variation in the daily output of urine; second, whether such injection will in any way affect the quantity of urine excreted and, if so, to find out if possible the factors involved.

LITERATURE

Magnus and Schäfer (1) working on anesthetized animals, concluded that intravenous injection of extracts of the pituitary gland causes a prolonged expansion of the kidney and a greatly increased rate of renal secretion. The diuresis was in every case of short duration, twenty to thirty minutes, while the kidney dilation continued for a longer time. These authors conclude that the extract acts directly on the renal epithelium in bringing about the increased flow of urine.

This work was later repeated by Schäfer and Herring (2) who arrived at practically the same conclusions. Their results, however, were quite inconstant. In a series of thirteen experiments on dogs, nine show

a diuretic and four an antidiuretic effect after injection. In another series on nineteen rabbits, diuresis was obtained in fourteen cases and a decrease in flow in five cases.

The theory of direct stimulation of the renal cells was also upheld by Hoskins and Means (3), who considered that the direct stimulation may be assisted by a vasodilation in the kidneys.

Houghton and Merrill (4) failed to find any direct action of pituitary extracts on the renal epithelium in the case of perfused kidneys. They conclude that diuresis is caused by an increase in the blood pressure.

Vasodilation of the renal vessels is considered by King and Stoland (5) to be the principal factor involved in the increased flow of urine which they find after pituitary extract injection.

Dale (6) working with perfused kidneys of the dog and cat found that pituitary extracts cause a vasoconstriction of renal vessels. These results were confirmed by Houghton and Merrill (4).

Pal (7) states that isolated rings of the proximal portion of the renal artery are constricted while rings from the peripheral portions of this artery are dilated by pituitrin.

The investigations of Falta, Newburg and Noble (8) indicate that diuresis generally results from subcutaneous injections of pituitary extracts.

A number of other investigators using practically the same methods as those of Schäfer and Herring have reported that diuresis results from the injection of extracts from the pituitary gland.

Within the past three years several investigators have reported that subcutaneous injection of pituitary extracts gives a diuresis of several hours duration. Pentimalli and Quercia (9) found that on the isolated kidney of the rabbit pituitary extract gave a diminished flow from both the ureter and from the renal vein. The decrease was especially marked in the flow from the vein. On the other hand Gabriels (10) reported that in the isolated kidney of the dog pituitary extract caused an increased flow of urine without vasodilation.

A large portion of the evidence in favor of the antidiuretic effect of posterior lobe extract comes from the clinical side where the extract has been used with apparent success in reducing the diuresis of diabetes insipidus. One of the first to report evidence of this nature was Farmi (11) who reported that he had been successful in reducing the diuresis in two diabetes insipidus patients by subcutaneous injections of extract of the pituitary.

Further evidence from the clinical side has been given by von der Velden (12), Konschegg and Schuster (13), Motzfeldt (14), (15) and Bab (16). Most of these investigators have checked up their results experimentally.

Meyenberg (17), working on rabbits and cats, found that subcutaneous injection produced an antidiuresis lasting for eight or ten hours.

Römer (18) found that animals catheterized every hour showed a very marked decreased output of urine after injection with pituitary extract.

The most extensive experimental work showing an antidiuretic effect from the injection of posterior lobe extract has been reported by Motzfeldt (19). Working with a large number of animals, mostly rabbits, he found that subcutaneous injection of the extract gave without exception a marked antidiuresis extending over several hours. Motzfeldt concludes that the results from his experiments on rabbits tend to show that pituitary extracts produce an antidiuretic action on account of their stimulation of the sympathetic nervous system and thus affecting the renal vasomotor system, that is, the direct cause of the antidiuresis is considered as due to vasoconstriction in the kidneys.

METHODS AND RESULTS

In all of our work we have followed rather closely the methods suggested by Motzfeldt with the important exceptions that our observations extended over much longer periods of time and the urinary output was always computed on the twenty-four hour or daily basis.

The experimental observations were made on cats and rabbits. Rabbits were found to be much more susceptible to pituitary extracts than were dogs or cats.

Three commercial extracts were used, namely, Pituitrin (Parke, Davis & Co.), Hypophyseal Solution (Squibb & Co.) and Pituitary Liquid (Armour & Co.). Practically no difference was found in the action of these preparations.

The injections were made subcutaneously in every case. The usual amount injected per day was 1 cc. for cats and 0.5 cc. for rabbits. The injections were made at the beginning of experiment at the time that the water was given by stomach tube.

Ordinary aseptic precautions were used in making the injections. No infections resulted from the repeated injections.

In order to obtain accurate data on the water intake the water was always given by stomach tube.

The animals were kept in perfectly dry cages and the nature and amount of food given were accurately noted in each case.

The urine was collected in such a way as to avoid so far as possible any evaporation or any contamination with feces.

The daily quantity and the specific gravity were the principal points noted in each experiment. Variations in the rate of output were also noted in the case of the catheterized rabbits.

Tests were made in each experiment for sugar and albumin but these were not observed. In both cats and rabbits the daily output of urine varies widely even with a constant food and water supply. On this account it was found advisable to extend the observations over several days.

In our first series of observations it was our purpose to find out whether pituitary extracts will, when injected subcutaneously, cause any variation in the daily output of urine. In table 1 we give the averages from this series of experiments. By inspection of this table it will be found that the averages for the control animals and for the injected animals are practically the same both in amount and in specific gravity. In six cases there is a slight decrease in the daily output of the injected animals but this is balanced by four cases where there is a greater increase in daily output. The variations above and below the mean were, with the exception of one case, less than 11 cc.

There is no indication that the animals established a resistance to pituitary extract after repeated injections. In practically every case the daily output of urine following the first and second injections was as high as that obtained on the ninth or tenth days of injections.

Attempts were made to study the effect of doses larger than 1 cc. per day, but these did not yield satisfactory results on account of the systemic disturbances caused. In the case of cats, vomiting was the most common result from large doses, in fact, even with 1 cc. doses a number of cats had to be rejected on account of this tendency to vomit following the injections. The vomiting may not occur until thirty or forty minutes after the water and the injections have been given. For this reason it is necessary to watch the animals closely for at least this length of time, so that one may be sure that he is not including regurgitated water in the urine measurements.

The apparent decrease in daily output of urine after injection shown in table 2 may be accounted for by the loss of water due to the increased defecation.

TABLE 1

Summary of experiments on the influence of pituitary extract on the urinary output in cats. The averages for each animal are computed from ten days of normal (control) and from ten days of daily injection with 1 cc. of pituitary extract. Each animal was given 100 grams of cooked meat per day. Water was given by stomach tube

		AMOUNT OF WATER GIVEN PER DAY	URINE	
			Average amount per day	Average specific gravity
		cc.	cc.	
A. Female; weight 2600 grams	Control	100	103.9	1014.5
	Pituitary extract	100	114.2	1011.3
B. Female; weight 2750 grams	Control	100	104.1	1013.8
	Pituitary extract	100	107.1	1013.3
C. Male; weight 2900 grams	Control	100	101.8	1025.6
	Pituitary extract	100	103.0	1024.8
D. Female; weight 2750 grams	Control	50	68.0	1021.2
	Pituitary extract	50	62.3	1019.8
E. Female; weight 2700 grams	Control	50	64.1	1027.4
	Pituitary extract	50	57.1	1030.0
F. Male; weight 2800 grams	Control	200	175.2	1008.6
	Pituitary extract	200	165.4	1010.4
G. Male; weight 2860 grams	Control	100	95.0	1015.4
	Pituitary extract	100	117.6	1016.0
H. Female; weight 2680 grams	Control	20	41.6	1044.0
	Pituitary extract	20	33.6	1040.0
I. Female; weight 2710 grams	Control	20	46.8	1044.0
	Pituitary extract	20	40.8	1045.0
J. Male; weight 2890 grams	Control	20	30.6	1043.0
	Pituitary extract	20	26.1	1045.0

In attempting to inject doses larger than 2 cc. per day no satisfactory results were obtained. In two cases the output was reduced to one-half the normal. In three cases there was a very marked increase in daily output. The usual result, however, was that it was impossible to keep the injected animals from regurgitating the water introduced by stomach tube.

Rabbits gave practically the same results as cats so far as the effect of pituitary extracts upon daily output and specific gravity of the urine is concerned. This is shown in tables 3, 4 and 5.

The variation between the control and the injected animals was greater in the experiments on rabbits than in those on cats. This is accounted for in part by the shorter time covered by the rabbit experiments. It was not possible to keep the rabbits in good condition for

TABLE 2

Effect of large doses of pituitary extracts on urinary output in cat. Pituitary extract was given in two doses per day of 1 cc. each at six hour interval. Animal was given daily 100 grams of cooked meat and 150 cc. of water by stomach tube

	DAY	URINE	
		Cubic centimeters	Specific gravity
Control	1	183	1010
	2	163	1010
	3	155	1010
Injected	4	108	1009
	5	173	1014
	6	165	1014

longer periods of experimentation than those used. In order to avoid the loss of excessive amounts of water in the large amount of feces passed, it was found advisable to reduce the food supply to such an extent that a very small amount of material was normally passed from the intestine. The reduced food supply was also found to be necessary to avoid the development of diarrhea following the injections.

Another cause for the variation in the case of rabbits may be found in the tendency of the pituitary extract to increase defecation. This fact is referred to later in another connection.

The second part of our problem was to find out whether the subcutaneous injection of pituitary extract causes any variation in the rate of urinary excretion. Rabbits were found to be best suited for this line of work since by using males it was easy to collect the urine at regular

intervals by catheterization. Silk linen catheters no. 11 were found to be best adapted for this purpose.

It was found necessary to select rabbits that could be catheterized with ease since irritation of the urethra apparently exerted a reflex inhibition on the kidney. Rabbit 3 in table 4 illustrates this point.

Tables 4 and 5 show the effect of pituitary extracts on the rate of

TABLE 3

Summary of experiments on the influence of pituitary extracts on daily urinary output in rabbits. The averages for each animal are computed from three days of normal (control) and three days with daily injection of 0.5 cc. of pituitary extract. Each animal was given 50 grams of cabbage per day, 150 cc. of water was given daily to each animal

		URINE	
		Average amount per day	Average specific gravity
		cc.	
5. Male; weight 1677 grams	Control	139.6	1012.6
	Pituitary extract	181.3	1018.0
6. Male; weight 1774 grams	Control	139.3	1013.3
	Pituitary extract	181.6	1014.0
7. Male; weight 1810 grams	Control	201.6	1016.6
	Pituitary extract	191.3	1011.6
8. Male; weight 1751 grams	Control	192.3	1007.3
	Pituitary extract	212.0	1013.6
9. Male; weight 1698 grams	Control	180.0	1010.0
	Pituitary extract	257.0	1012.3
10. Male; weight 1830 grams	Control	227.6	1010.0
	Pituitary extract	187.6	1012.0

secretion, but this is even better shown in figure 1. It will be noted from the figure that the normal diuresis which follows the giving of 150 cc. of water to rabbits by stomach tube reaches its maximum in the second or third hour after giving the water. By the end of the seventh hour the diuresis has practically exhausted itself and the normal excretion of 2 to 3 cc. per hour follows. After the injection of pituitary extract the picture is quite different.

In this case the diuresis is held in check for seven or eight hours when it breaks through and may reach a level as high as that of normal secretion. As a rule the diuresis following the injection is prolonged for ten to twelve hours. The total output per day is found to be practically the same in both cases.

By referring to some of Motzfeldt's (15) figures it will be noted that they show an increase in urine output in the seventh and eighth hours,

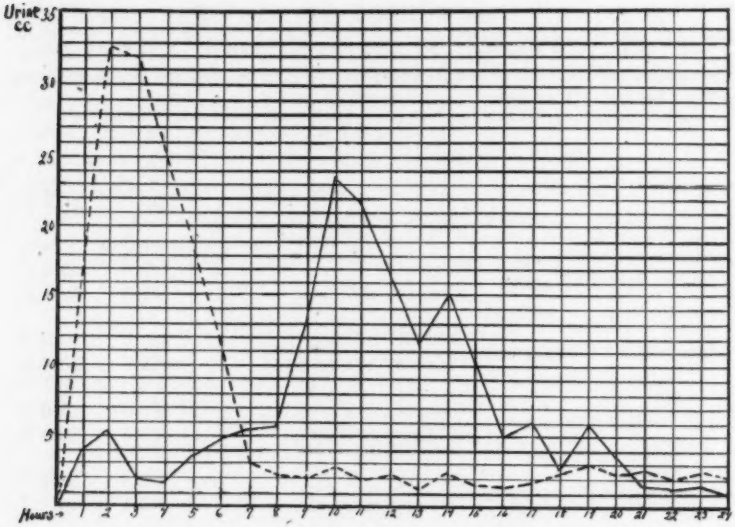

Fig. 1. Curves showing the effect of pituitary extract on urinary output. Each curve is plotted from averages from two animals (rabbits). At the beginning of the experiment each animal was given 150 cc. of water per os. The injected animals were each given 0.5 cc. of pituitary extract subcutaneously at the beginning of the experiment. The broken line represents the control animals. The continuous line represents the injected animals. Average 24 hours urinary output of control animals, 172.6 cc. Average 24 hours urinary output of injected animals, 170.5 cc.

that is, his observations were carried just to the beginning of the diuresis which has been delayed by the pituitary extract.

The third problem which concerned us was the cause of the delay in diuresis which we found followed the subcutaneous injections of pituitary extract. Several things which appeared in the course of the

experiments suggested to us that delayed absorption from the alimentary canal may be an important factor in causing this delayed diuresis. It has already been noted that in the case of injected cats there is a pronounced tendency to vomit. The vomiting may occur three-quarters of an hour after giving the water and the injection. Usually four- to five-sixths of the water was returned in the vomit, indicating that there

TABLE 4

Effect of pituitary extract on the urinary output in rabbits. Each animal was given 150 cc. of water by stomach tube at the beginning of the experiment. No food was given during the experiment. Rabbits 1, 2 and 3 were injected at the beginning of the experiment with 1 cc. of pituitary extract. The urine was drawn by catheterization

	(1) Male; weight 1720 grams pituitary extract urine	(2) Male; weight 1780 grams pituitary extract urine	(3) Male; weight 1675 grams pituitary extract urine	(4) Male; weight 1930 grams control urine
	cc.	cc	cc.	cc
6.00 a.m.				
6.30 a.m.	5.0	3.0	2.5	2.5
7.00 a.m.	20.0	4.0	4.0	5.0
7.30 a.m.	15.0	2.0	2.0	13.0
8.00 a.m.	4.0	1.5	2.0	13.0
8.30 a.m.	4.5	3.5	1.5	19.0
9.00 a.m.	0.5	5.0	1.0	18.0
9.30 a.m.	4.0	10.0	1.0	16.0
10.00 a.m.	9.0	5.0	1.5	0.5
10.30 a.m.	4.0	4.0	1.5	24.0
11.00 a.m.	0.5	2.0	2.0	7.0
11.30 a.m.	1.0	2.0	0.5	0.0
12.00 m.	3.5	2.5	1.5	5.5
Total first 6 hours.....	71.0	46.0	20.0	123.5
6.00 p.m.	56.5	57.5	11.5	18.5
6.00 a.m.	91.0	48.0	51.0	31.0
Total 24 hours.....	218.5	151.5	82.5	173.0

was a delay in passing the water from the stomach. The injection of large doses of the extract commonly caused diarrhea. The effect of the extract on the alimentary canal was much more pronounced in rabbits than in cats. Rabbits kept on a uniform diet of 50 grams per day of carrots or cabbage pass very small amounts of feces in the form of comparatively dry pellets. After injection with 0.5 cc. of pituitary extract there is a marked increase in the amount of feces which are of a

TABLE 5

Periodic variation in output of urine in male rabbits following injection of pituitary extracts. Each animal was given daily 50 grams of carrots and 150 cc. of water by stomach tube. The water and the pituitary extract were given at the beginning (6 a.m.) of the experiment. Injected animals received 0.5 cc. of pituitary extract subcutaneously

		DATE	TIME	URINE		Total amount urine per day
				Cubic centimeters	Specific gravity	
				cc		
11. Weight 1934 grams	Control	May 29–30	12 m.	101.0	1010	
			6 p.m.	0.0		
	Pituitary extract		6 a.m.	103.0	1006	204.0
		May 30–31	12.00 m.	34.0		
			6.00 p.m.	15.0		
			6.00 a.m.	95.0	1010	154.0
12. Weight 1677 grams	Control	May 29–30	12.00 m.	122.0	1005	
			6.00 p.m.	16.0		
	Pituitary extract		6.00 a.m.	30.0		154.0
		May 30–31	12.00 m.	18.5		
			6.00 p.m.	29.0		
			6.00 a.m.	134.0	1009	181.5
13. Weight 1740 grams	Control	May 29–30	12.00 m.	108.0		
			6.00 p.m.	17.0		
	Pituitary extract		6.00 a.m.	42.0		167.7
		May 30–31	12.00 m.	40.0	1004	
			6.00 p.m.	0.0		
			6.00 a.m.	45.0	1015	85.0
14. Weight 1734 grams	Control	May 29–30	12.00 m.	133.0	1004	
			6.00 p.m.	12.0		
	Pituitary extract		6.00 a.m.	38.0		183.0
		May 30–31	12.00 m.	37.0		
			6.00 p.m.	88.0	1002	
			6.00 a.m.	95.0	1010	220.0
15. Weight 2085 grams	Control	May 29–30	12.00 m.	158.0	1005	
			6.00 p.m.	17.0		
	Pituitary extract		6.00 a.m.	11.0		186.0
		May 30–31	12.00 m.	23.0		
			6.00 p.m.	30.0		
			6.00 a.m.	133.0	1007	186.0

TABLE 5—*Continued*

		DATE	TIME	URINE		Total amount urine per day
				Cubic centimeters	Specific gravity	
						cc.
16. Weight 2027 grams	Control	May 29–30	12.00 m.	145.0	1006	
			6.00 p.m.	27.0		
			6.00 a.m.	16.0		188.0
	Pituitary extract	May 30–31	12.00 m.	35.0		
			6.00 p.m.	43.0	1015	
			6.00 a.m.	87.0	1015	165.0
17. Weight 2285 grams	Control	May 29–30	12.00 m.	145.0	1008	
			6.00 p.m.	25.0		
			6.00 a.m.	5.0		175.0
	Pituitary extract	May 30–31	12.00 m.	43.0		
			6.00 p.m.	11.0		
			6.00 a.m.	133.0	1012	187.0
18. Weight 2640 grams	Control	May 29–30	12.00 m.	135.0	1008	
			6.00 p.m.	63.0		
			6.00 a.m.	35.0		234.0
	Pituitary extract	May 30–31	12.00 m.	42.0	1024	
			6.00 p.m.	2.0		
			6.00 a.m.	123.0	1012	167.0

semifluid consistency. This excessive amount of water feces accounts for the apparent decrease in the daily urinary output found in some cases after injection. An example of this is seen in rabbits 13 and 18 in table 5.

If rabbits are injected with doses of 1 cc. or more, large masses of semi-fluid feces are thrown off in five to ten minutes after giving the water and the injection. The large amount of water given was not the direct cause of the diarrhea since the controls passed small amounts of feces in the usual pellet form.

The effect of pituitary extracts on absorption was tested experimentally. The results indicate that there is a retarding of absorption after subcutaneous injection of the extract.

In order to avoid the possible effect of the anesthetic on the rate of absorption, a decerebrated dog was used in the following experiment.

When a diuresis was produced by the constant intravenous injection with the Woodyatt injection apparatus of 150 cc. of 0.9 sodium chloride

TABLE 6

Effect of pituitary extract on intestinal absorption in the cat. The animal was anesthetized lightly by giving urethane (2 grams per kilo) per os. The small intestine was exposed and washed, then ligated at either end

	AMOUNT OF WATER INJECTED INTO SMALL INTESTINE	WATER RECOVERED AFTER 1 HOUR	ABSORPTION
	cc.	cc.	cc.
Control animal	30	8	22
Injected animal 1 cc. pituitary extract, subcutaneously	30	27	3

solution per kilo per hour, the subcutaneous injection of pituitary extract had no effect on it (three experiments). On the other hand the injection of the extract caused a delay in the diuresis caused by giving 150 cc. of 0.9 salt solution by stomach tube.

The fact that the rate of diuresis caused by giving 0.9 NaCl solution intravenously is not affected by subcutaneous injection of pituitary extracts but is affected by such injections when the salt solution is introduced into the alimentary canal would indicate that the extract in some way causes delayed intestinal absorption.

TABLE 7

Effect of pituitary extract on intestinal absorption in the rabbit. Anesthetized and intestine prepared as in table 6

	AMOUNT OF WATER INJECTED INTO SMALL INTESTINE	WATER RECOVERED AFTER	ABSORPTION
	cc.	cc	cc.
Control animal	30	0	30
Injected animal 0.5 cc. pituitary extract subcutaneously	30	25	5

TABLE 8

Effect of pituitary extracts on intestinal absorption in a decerebrated dog. A 14 inch loop from the middle of the jejunum was used

	AMOUNT OF WATER INJECTED INTO INTESTINE	WATER RECOVERED AFTER 30 MINUTES	ABSORPTION
	cc.	cc.	cc.
Control period	75	42	32
Injected period	75	70	5

The fact that pituitary extract does not affect the diuresis following intravenous injection of normal salt solution would also indicate that the extract does not regulate the diuresis by an action on the salt content of the blood as has been recently advocated by Abrahamson and Climenko (20).

TABLE 9

Effect on the diuresis produced by giving 150 cc. of 0.9 NaCl solution per os in rabbits

		DATE	TIME	URINE		TOTAL AMOUNT URINE PER DAY
				Cubic centimeters	Specific gravity	
						cc
Rabbit 19; male; weight 2100 grams	Controls	July 25–26	12.00 m.	86	1013	
			6.00 p.m.	28		
			6.00 a.m.	42		156
		July 26–27	12.00 m.	122	1011	
			6.00 p.m.	51		
			6.00 a.m.	47	1019	220
		July 27–28	12.00 m.	82	1018	
			6.00 p.m.	94		
	Pituitary extract 0.5 cc. per day		6.00 a.m.	90		266
		July 28–29	12.00 m.	58		
			6.00 p.m.	55		
			6.00 a.m.	77	1013	190
Rabbit 20; male; weight 1550 grams	Controls	July 25–26	12.00 m.	182	1010	
			6.00 p.m.	58		
			6.00 a.m.	52	1024	292
		July 26–27	12.00 m.	114	1015	
			6.00 p.m.	70		
			6.00 a.m.	61	1025	245
		July 27–28	12.00 m.	97	1012	
			6.00 p.m.	63		
	Pituitary extract 0.5 cc. per day		6.00 a.m.	122	1022	282
		July 28–29	12.00 m.	90		
			6.00 p.m.	47		
			6.00 a.m.	73	1025	210

Previous workers have shown that subcutaneous injection of pituitary extracts does not affect the general blood pressure in anesthetized animals. We found that in decerebrated animals subcutaneous injection of the extract had no effect on blood pressure (two experiments). The effect of the extract on the rate of urinary excretion can not then be due to any general vasomotor change. It is possible, however, that

there may be a vasoconstriction in minute vessels of the intestinal wall thus causing a delayed absorption and incidentally a delay in the diuresis. This does not necessarily rule out the possibility that there may also be a simultaneous vasoconstriction in the kidney which is also a factor in retarding the diuresis.

CONCLUSIONS

1. Subcutaneous injections of pituitary extract do not alter quantitatively the daily output of urine in cats and rabbits, nor do they cause any marked variation in the specific gravity of the urine.

2. The subcutaneous injection of pituitary extracts causes a delay of seven to eight hours before the beginning of the diuresis which follows the ingestion of large amounts of water. This delay, however, does not cause any variation in the total amount of urine excreted in twenty-four hours.

3. The delay in diuresis which is produced by subcutaneous injection of pituitary extract is due in part at least to a delayed absorption from the alimentary canal.

4. The subcutaneous injection of pituitary extract has no influence on the diuresis induced by a continuous intravenous injection of isotonic salt solution.

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THE INITIAL AND PROGRESSIVE STAGES OF CIRCULATORY FAILURE IN ABDOMINAL SHOCK

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INTRODUCTION

Although the circulatory failure in shock has been most extensively studied, it appears that the sequence of dynamic events, particularly in the earlier stages, has not been investigated with the degree of detail possible by optically recording manometers. Their use offers the advantages that, without resorting to extensive operative procedures, the pressure changes in the auricles, ventricles and arteries may be accurately recorded. With the knowledge gained from foregoing work (1) as to how individual factors of the circulation can modify the details of the optical pressure curves, it is possible by studying their modification during the progress of circulatory failure in shock to determine more minutely than in other ways the dynamic stages taking place in the circulation from time to time.

Since "exposure of the intestines" is generally considered the method "par excellence" for producing experimental shock and therefore the procedure most frequently employed, the study of the dynamic sequence in circulatory failure so produced is alone considered in this investigation.

EXPERIMENTAL PROCEDURE

The fundamental aim of this research was to analyze the consecutive changes evident in optical records of the right auricular, right ventricular and central arterial pressures. As it is undesirable, however, to take continuous photographic records of these pressure changes, the mean carotid pressure was constantly recorded on smoked paper as a rough index of the circulatory changes and the "effective venous pressure" was read at shortly spaced intervals from a differential water-manometer. Such a manometer is obtained when the fluid-filled limb

of an ordinary water-manometer is connected with a sound inserted via the jugular vein into the auricle and the air column of the other limb is connected with a trocar inserted into the thoracic cavity.[1] At stated intervals, marked on the smoked drum by a signal electrically connected to the photokymograph when the shutter opens, 50 cm. of pressure tracings were optically recorded.

After a period of preliminary observation under light ether anesthesia, abdominal shock was induced in the following way: A long median abdominal incision was first made through the skin and connective tissue. After studying the vascular reaction thus induced, the incision was extended through the muscles and peritoneum. Finally, the intestinal loops were removed and spread upon the abdomen and a stream of moist air allowed to blow over them, during the reminder of the experiment. Experience showed that circulatory failure is more evenly produced without manipulation of the intestines and when this is indulged in it acts only to complicate the dynamic stages.

ANALYSIS OF RESULTS

The progress of the circulatory failure, as expressed by the changes in mean arterial pressure and effective venous pressure, by the changes in heart rate and respiration, are shown in a typical experiment plotted

[1] *Correction.* While casual consideration led to the impression that this type of manometer would offer a simple and direct method of reading the effective venous pressure in absolute terms, more careful reflection, together with subsequent experimental controls, shows that the effective venous pressure cannot be measured absolutely in this way. This is due, briefly, to the fact that the pleural cavity is virtual and not real; consequently the limited quantity of air in the connecting tubes suffers compression and rarefaction as the fluid level in the water manometer changes with the intra-auricular pressure. This makes the reading of the differential manometer considerably higher than the difference actually existing between the pressures in the pleural cavities and in the auricle. It has been found, however, that as long as intrapleural pressure variations do not change excessively, due to modified breathing, the figures so obtained follow the directional changes of effective venous pressure although they are not proportional to them. Inasmuch as these conditions obtain in all the experiments reported in this investigation, the values plotted, though neither absolute nor proportional as regards changes in effective venous pressure, are reliable as regards its directional tendency. This error is unfortunate in the fact that the absolute variations of effective venous pressure during circulatory failure are not available. Since the directional tendency is correctly indicated, however, it does not invalidate the essential conclusions in regard to the dynamics of the circulation in this form of circulatory failure here described.

in figure 1. The changes are in accord with those generally recognized as characteristic of circulatory failure in shock.

The onset of circulatory failure. A glance at the results presented in this chart shows that while the preliminary stage of the experiment involving the abdominal incision and removal of the intestines produces temporary reactions and often leaves the arterial and venous pressures somewhat low, the changes are not sufficient to be considered as even an initial stage of circulatory failure. After these operative stages have been completed, tracings of the auricular, ventricular and carotid pres-

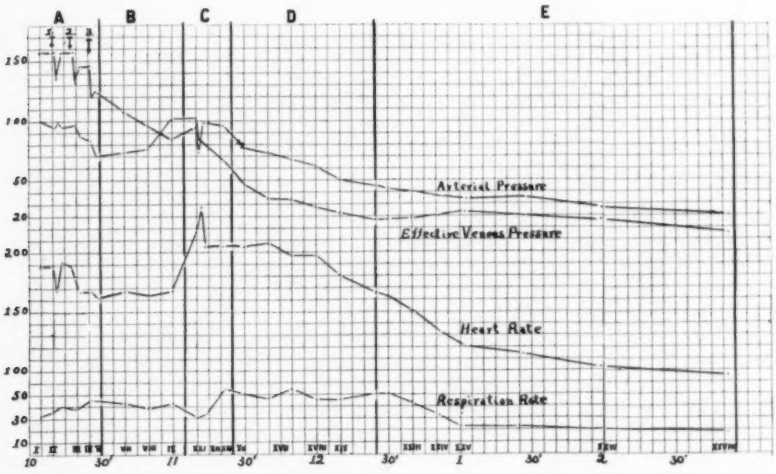

Fig. 1. Chart showing plotted data of circulatory stages in a case of abdominal shock (exp. C-157); *A*, operative stage; *B*, initial stage; *C*, effect of handling intestine; *D*, progressive stage; *E*, complete circulatory failure. Roman numerals in lower spaces refer approximately to times when optical records shown in figure 2 were recorded.

sures retain all the details that we regard as typical of normal dynamic conditions of the circulation (fig. 2, *I* to *IV*).

The reactions induced by the preliminary operative procedures are therefore physiological in nature and probably no greater than occur in many reflex effects on the circulation in everyday life. Furthermore, vigorous and repeated blows upon the abdomen with a wooden mallet were found to initiate no more than a temporary disturbance of the vascular system in every respect similar to that produced by opening

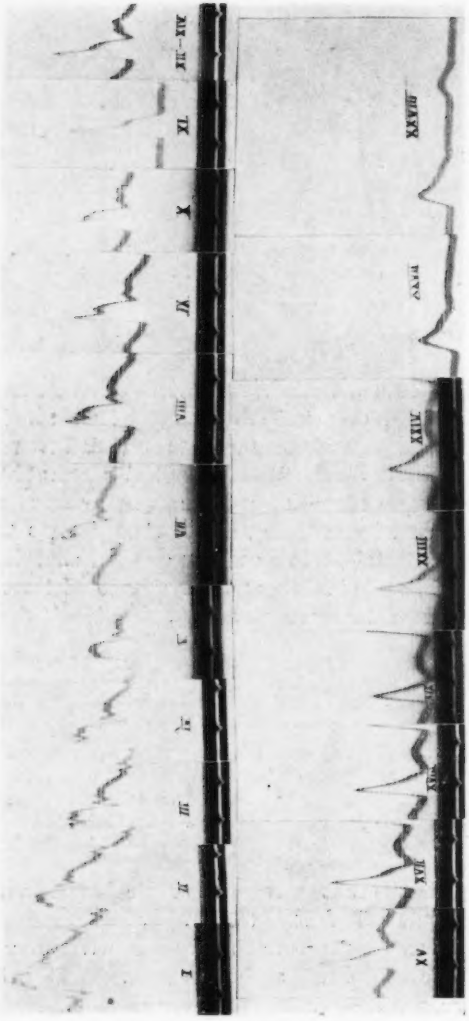

Fig. 2. (Three-eighths actual size). Segments of optical arterial tracings taken during progressive failure of the circulation in shock (exp. C-157). Numerals correspond to those in figure 1 so that relations to other events can be studied.

the abdomen and never followed by a condition that could by any stretch of the imagination be referred to as "circulatory failure." *Circulatory failure in abdominal shock, therefore, sets in only after the intestines have been exposed to the air for some time.* In the detailed analyses of records, however, we must consider carefully the possible bearings of these preliminary reactions upon the subsequent development of shock.

Effect of operative procedures on the circulation. Under light anesthesia the first incision through the skin and fascia of the abdominal wall produces a temporary cessation or diminution of the respirations, associated with a temporary fall of arterial pressure. The effective venous pressure is unaffected. From this reduced state of arterial pressure recovery is complete. Upon then extending the incision through the

Fig. 3. (One-half actual size). Three segments of optical arterial tracings taken before (*I*), during (*II*) and after abdominal incision (*III*). (exp. C-152), showing the effect of cardiac inhibition on pressure variations: *a*, *b*, preliminary vibrations; *b*, *c*, *d*, primary vibration; *d*, *e*, systolic summit; *e*, *f*, incisura; *f*, *g*, after-vibrations; *g*, *h*, diastolic period.

abdominal muscles and peritoneum the arterial pressure again falls in a similar manner but does not recover completely. The effective venous pressure is often lowered slightly (fig. 1).

An analysis of the kymograph records (fig. 1) corroborated by the more exact optical tracings of the carotid pressure (fig. 3) shows that the predominant cause of the blood pressure decline was a moderate reflex cardiac inhibition which was temporary after the skin incision but often became permanent after the peritoneal incision. The slight decrease in venous pressure was no greater than could be directly accounted for by the removal of the intra-abdominal pressure upon the abdominal veins and it is very doubtful whether the venous pressure

was sufficiently impaired to affect the systolic discharge appreciably. As a rule, this reflex cardiac inhibition left the arterial pressure at a level not far from but somewhat below normal, but in two instances it became so marked that the pressure fell very nearly to the critical level. It should be added that this reflex inhibition was obtained only when the heart rate was rapid on account of the anesthesia and was absent whenever the heart was already slow, due to high vagal tonus (e.g., after morphine). Nor was it present when the reflex arc was depressed either at the vagal terminals by atropine or centrally by deep anesthesia. It is therefore questionable whether this reflex inhibition comes into play at all in man when vagal control is already established. We may accordingly begin our analysis as to the real initiation of the circulatory failure in shock at this stage of the experiment.

Initial stages of circulatory failure in abdominal shock. Upon continued exposure of the intestines to the air, circulatory failure is initiated. As shown in the chart of figure 1, the first gross dynamic change occurred within the first half hour and consisted in a slight fall of mean arterial pressure. During this time the effective venous pressure remained unchanged or increased gradually if the cardiac rate remained unaltered (fig. 1). A careful study of the optical arterial tracings taken during the first half hour following exposure of the intestines, indicates clearly that this must be considered as the initial stage of circulatory failure even though the effective venous pressure does not alter or even increases and the mean pressure has fallen only to a small extent (Cf. note 2, page 493). These changes are well shown in segments *V* to *IX* of figure 2 which correspond to numerals indicated on the chart of figure 1. Owing to the larger amplitude of curves, they are shown even in better detail in the segments of another experiment reproduced in figure 4. In this experiment, twenty-four minutes after the intestines were removed the venous pressure was practically equal to that before removal and the arterial pressure had fallen only from 128 to 108, not an abnormal level of mean pressure. A comparison of the last three segments, taken at ten minute intervals, with the first segment obtained immediately after removal of the intestines, shows clearly that a definite reaction had been inaugurated in the vascular system. The preliminary vibration (*a* to *b*) has practically disappeared; the primary oscillation (*b*, *c*, *d*) is much larger and the systolic summit (*d* to *e*) gradually changes from an ascending to a horizontal and then to a descending plateau. The pressure at the beginning of diastole (*g*) is much lower and, in spite of the lower pressure level, the gradient of

the diastolic limb (*g*, *h*) is much steeper. *Such signs are indicative of a diminished distention of the large arterial trunks.* It is evident that the actual level of the mean pressure, as recorded by a damped mercury manometer, is no indicator of the important dynamic changes that have

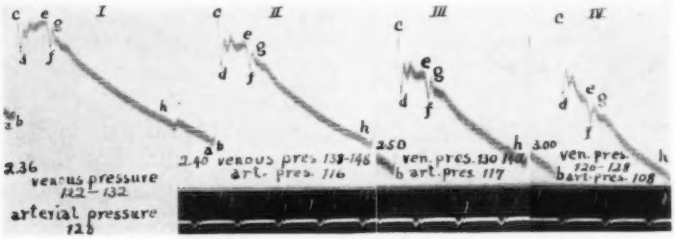

Fig. 4. (One-third actual size). Four segments of optical arterial tracings showing essential changes in arterial pressure variations in initial stage of circulatory failure when mean arterial pressure had fallen only slightly. Letters the same as before (exp. C-156): *I*, normal; *II*, after opening abdomen and intestinal exposure; *III*, ten minutes later; *IV*, twenty minutes later.

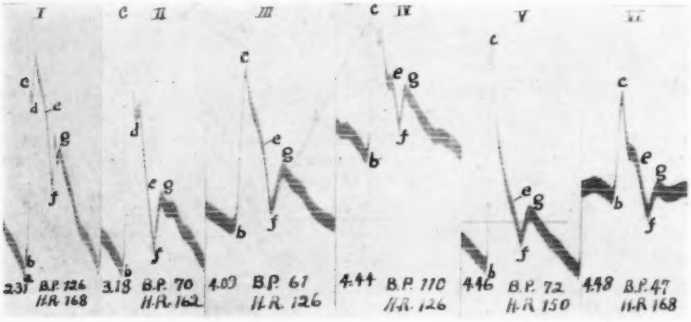

Fig. 5. (Two-fifths actual size). Segments of optical arterial tracings during initial and progressive stages of shock (exp. C-147). Letters same as before: *I*, after opening abdomen; *II*, during initial stage; *III*, during progressive stage; *IV*, after elevating animal board; *V*, immediately after 2.5 grains sodium nitrite; *VI*, two minutes later.

already taken place. Evidently, two factors tend to keep the mean pressure up in this case—the greater fling of the blood column, as indicated by the primary wave, and the abbreviation of diastole due to the cardiac acceleration.

Similar results are obtained in other conditions. Thus, when the aortic valves are suddenly rendered incompetent, the mean pressure sometimes undergoes no alteration or at most a very slight fall in spite of the most pronounced alterations indicated in the optical curves. When amyl nitrite is inhaled the mean carotid pressure may show no alteration or a rise, observations that have erroneously been attributed either to an irregular reaction of the animal by vasoconstriction or to the use of inferior drugs. Optical records show evidence of distinct vasodilation. In these cases also the mean pressure is prevented from falling by the same factors as we have in shock—the cardiac acceleration and the greater throw of the blood column in the less distended arteries.

Two factors may account for the diminished distention of the arterial trunks in the early phase of circulatory failure—a decreased total resistance and a reduced minute output of the left ventricle. In favor of the former are (1) the observation of the engorged intestinal vessels; (2) the more rapid decline of the diastolic limb of the pulse curve even

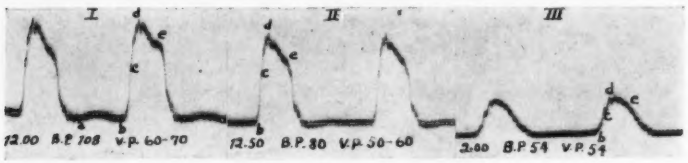

Fig. 6. (Two-fifths actual size). Segments of optical right intraventricular pressure curves in different stages of circulatory failure: *a*, *b*, auricular systole; *b*, *c*, isometric period; *c*, *e*, systolic ejection phase (exp. C-160). *I*, normal before operation on abdomen; *II*, after exposure and manipulation of intestines, initial stage; *III*, late portion of progressive phase.

though the pressure at the onset of diastole is much below normal; and (3) the fact that identical curves are obtained after the use of nitrites. Against the latter assumption are the high and often unaffected effective venous pressure and the accelerated heart rate which, on the basis of Henderson's work (2), may be anticipated to augment the minute output. Furthermore, the gradient of the ascending limb of the intraventricular pressure curve, which occurs during the isometric phase of cardiac contraction, remains unaltered even when venous pressure has actually become somewhat reduced. This is well shown in the first two segments of figure 6. In this experiment, fifty minutes after exposure of the intestine the right ventricular pressure curves, though smaller in amplitude, show no alteration of the gradient of the ascending limb (*b* to *c*) and the semilunar valves open (*c*) at the same level from the

base line. Only the slope of plateau (*d*, *e*) is modified. Yet at this time the effective venous pressure had fallen about 10 mm. of saline.

There is, therefore, no evidence that the diminished distention of the arterial trunks, which produces but a slight reduction of the mean pressure but pronounced changes in the optical tracings, is due to a decreased minute output of the heart, but distinctly favors the view that circulatory failure is initiated by reduced total resistance, presumably in the abdominal vessels.

The thought at once occurs to one: How is it possible, with a reduction of peripheral arterial resistance and the consequent accumulation of blood in the abdominal vessels, that the effective venous pressure can remain normal? Yet this is apparently the case, not only in the early stages of abdominal shock but in the most intense vasodilation it has been possible to produce by the use of nitrites. Augmented breathing and greater negative pressure variation within the thorax might be thought of, did this not happen even when respirations are entirely unchanged or are smaller and slower.

A record of the intra-auricular pressure in such experiments shows that the stability of the venous pressure is virtual and not real. It indicates, moreover, that an even finer conception of the relation between venous pressure and ventricular ejection is necessary than that stated by Henderson and Barringer (3). The differential water-manometer, as used, is a mean-pressure recording device capable of indicating only the respiratory variations with any degree of accuracy. The pressure in the larger veins and auricles, like the arterial pressure is never constant but is always changing.[2]

The normal variations occurring in the auricle have been recorded by optical manometers and described by Piper (4), Garten and Weber (5) and the writer (6). They are also shown in segment *I*, figure 7. The pressure both rises and falls during auricular systole (*a*, *b*, *c*) and the fall is continued into that portion of auricular diastole comprised by the inter-systolic interval (*c*, *e*). With the onset of ventricular contraction (*d*) the pressure is abruptly elevated or lowered, differing

[2] The fact may be emphasized that mean pressure does not exist in any part of the vascular system. Theoretically it is a mathematical average of the individual pressure fluctuations. Practically, it is the pressure recorded by a very inefficient manometer which unfortunately rarely agrees with our theoretical figure. In the arteries it bears a variable relation to the systolic and diastolic pressures, depending on many circulatory factors which it is hoped some day to make the subject of a separate communication.

apparently in different animals. During the period of ventricular contraction (*d*, *e*) the pressure rises slowly and during the ventricular diastole it falls more or less until the next auricular systole. *It is the height of the effective pressure at the moment that the a–v valves open* (*e*) *which determines the diastolic ventricular filling; while the depth to which the pressure falls, in the common auriculo-ventricular cavities, just previous to ventricular systole* (*d*) *determines the volume and force of blood ejected by the ventricles, tersely expressed as the ventricular efficiency.* If we follow the changes in auricular pressure during the early stage of shock (segments *I* to *IV*, figure 7) it is found that exposure of the intestines alone reduces the return-flow of blood, as indicated by the

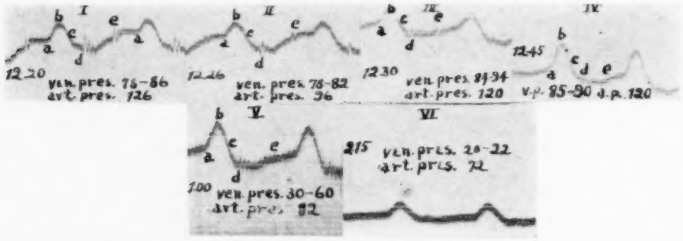

Fig. 7. (Two-fifths actual size). Segments of optical curves of right auricular pressure during different stages of circulatory failure (exp. C-158); *a*, *b*, *c*, auricular systole; *c*, *d*, intersystolic period; *d*, *e*, ventricular systole; *e*, *a*, ventricular diastose; *I*, normal; *II*, after skin incision; *III*, after intestinal exposure, initial stage; *IV–V*, progressive stages of shock; *VI*, complete circulatory failure.

slower rise of pressure from *d* to *e* (segment *III*). This is more than counteracted, however, by a greater pressure in some manner produced by auricular contraction (*a*, *b*) in consequence of which not only the general level of the curve is higher but the effective pressure and the initial pressure for ventricular contraction (*d*) is also higher. As long as the volume of blood returned to the auricles is not reduced too much these compensatory mechanisms are able to counteract the effects that would otherwise be produced by a deficient return of blood. By studying the finer details recorded by optical apparatus we can appreciate why the mean effective pressure can remain unaltered or even increase in spite of the fact that the return-flow of blood is partially reduced.

The stage of progressive circulatory failure. The stage of progressive circulatory failure extends from the initial stage to the time that the

mean pressure passes below 50 mm. of mercury. This figure has been arbitrarily selected by most experimenters as critical because it is difficult, by known methods, to restore the pressure to normal for any length of time when it has fallen below this level. The duration of this stage is variable, extending, in my experience, from thirty minutes to four hours. The general features are shown in the experiment plotted in figure 1. In this experiment the arterial pressure fell within one and one-half hours after intestinal exposure to 50 mm. This was accompanied by a rapidly progressing fall of the effective venous pressure. The heart accelerated. This is typical when vagus tonus is present at the beginning. The respirations increased in rate and amplitude.

The optical arterial pressure curves recorded during this stage become progressively smaller in amplitude and the features characteristic of a great depletion of the arterial trunks become more pronounced. This transformation is shown in segments *X* to *XIX*, figure 2, taken from the same experiment that is plotted in figure 1. They are also shown in the six segments of figure 5. If we compare segment *III* of this figure taken during the stage of progressive failure, with the normal, shown in segment *I*, we find that the complex central arterial pressure curve, normally present, is transformed to a curve with a very simple contour. The incisura (*e*, *f*) is less abrupt and is followed by a slow instead of a sharp rise (*f*, *g*) which results in the appearance of a dicrotic wave in the central pulse.

Recovery of the mean pressure, and in a certain measure of the typical form of the pressure tracing, was attained by increasing the venous pressure by tilting the animal board upward at an angle of approximately 30°. The changes are shown in figure 5, segment *IV*. When the pressure was thus restored, 2½ grains of sodium nitrite were given intravenously. Thereupon the mean pressure fell to 47 mm. mercury and the heart accelerated. The pulse contour during the early period of the pressure-fall is shown in figure 5, segment *V*. It is characterized by a rapid rise, mounting to a sharp peak; followed by a rapid systolic decline. The incisura (*e*, *f*.), which can scarcely be differentiated from this fall, is again followed by a dicrotic wave. During the depth of the mean arterial pressure fall the chief characteristics of the previous optical tracing are retained, except that the amplitude is smaller and the incisura deeper. The pressure line during the short diastole is practically horizontal (fig. 5, seg. *VI*).

The facts (1) that the mean pressure and the normal contour of the central pulse can in a measure at least be restored by increasing venous pressure, and (2) that the peripheral vessels are still capable of dilating in reaction to vasodilator drugs, indicate that the progressive deficiency in the arterial volume during this stage is predominantly due to low venous pressure. This is also corroborated by the changes in intra-auricular and intraventricular curves obtained during this stage of circulatory failure. As seen in figure 7, segments *V* and *VI*, the pressure within the auricle rises less and less during ventricular systole, finally remaining absolutely horizontal except when auricular contraction causes an elevation. In consequence, the initial pressure within the ventricle decreases and the intraventricular pressure curve, as shown in figure 6, segment *III*, shows a small gradual rise during the isometric period and a rounded contour during the ejection period, both characteristic of low initial pressure (1).

Stage of complete circulatory failure. When complete circulatory failure had been established the effective venous pressure was extremely low; the heart began to slow and thereby further reduced the minute output of the left ventricle. The impression is gained from these experiments that this is the final cause of circulatory failure in shock.

The progressive changes in the optical arterial tracings during this stage are shown in figure 2, segments *XIX*, *XXVIII*. The pulse form is always of a very simple type and resembles the peripheral pulse in normal animals. The rise is more gradual and the pressure falls markedly during the later systolic period. There is no sharp incisura and the pressure at the beginning of diastole is very low and decreases very little during the diastolic period. Evidently, the peripheral flow entirely ceases during diastole and is limited to the period of systole.

Effect of handling the intestines. The sequence of dynamic changes here discussed follows when handling of the intestines during the course of the experiment is reduced to a minimum. The act of manipulation was found to cause a variety of reactions in different animals. In some cases, as charted in figure 1, it caused a temporary reduction of arterial pressure accompanied by a decreased venous pressure. The optical arterial pulse shows an essential difference in that, during the diastolic period, the pressure line remained practically horizontal (fig. 2, cf. seg. *X* and *XI*). From this state the mean arterial pressure, as well as the contour of the pulse curve, recovered but the venous pressure often remained low. Apparently, the dynamic changes are associated with direct vascular effects produced by mechanical irritation. Hand-

ling was sometimes accompanied by reflex cardiac inhibition, in which case the mean arterial pressure fell markedly without any alteration of the effective venous pressure. Such effects were temporary, as a rule, and did not persist even when handling was long continued. In other cases intestinal manipulation caused an increase in rate and depth of respiration and a marked elevation of arterial pressure due to a removal of cardiac inhibition and consequent acceleration of the heart.

SUMMARY AND DISCUSSION OF RESULTS

The course of the circulatory failure in abdominal shock may be divided into three stages:

1. *The initial stage*, lasting about thirty minutes after intestinal exposure, during which effective venous pressure and cardiac discharge are apparently not appreciably reduced but the arterial pressure, as recorded precisely shows distinct alterations not adequately indicated by mean pressure manometers.

2. *The progressive stage*, lasting two to four hours, during which effective venous pressure falls progressively, cardiac efficiency is impaired and the arterial pressure falls toward a low level. The heart usually accelerates.

3. *Complete circulatory failure*, marked by a prolonged period during which effective venous pressure has reached its lowest level and the arterial pressure slowly falls further until death supervenes. During this stage cardiac slowing usually takes place.

A careful study of the optical tracings of arterial, intraventricular and auricular pressures, accompanied by constant readings of the mean arterial and effective venous pressures during these stages, corroborates the conclusion—in the ascendency at the present time—that *the decreased venous pressure and consequent reduction in minute output is the predominate factor in the pronounced fall of arterial pressure during the progressive stage of shock*. The dynamics of the circulation indicate clearly, however, that *a reduction in peripheral arterial resistance initiates the fall of arterial pressure and the diminished filling of the arterial trunks* before the effective venous pressure and cardiac discharge are reduced.

The rôle that a diminished arterial resistance plays in the circulatory failure is therefore directly established for the first time. Hitherto, evidence regarding the state of peripheral resistance in shock has necessarily been inferred from the state of the peripheral vessels.

Although vasodilation following vasomotor exhaustion has been suggested as a cause of circulatory failure in shock by Keen, Weir Mitchell and Morehouse (7) in 1864 and received experimental support from Fisher (8) in 1870 and from Crile (9) in 1899, the bulk of subsequent work has failed to confirm the hypothesis that peripheral resistance in shock is reduced. The experiments of Seelig and Lyon (10), of Seelig and Joseph (11), Morrison and Hooker (12), Muns (13), Guthrie (14) and others, in fact, indicate that the resistance in the peripheral vessels, on the contrary, is increased. . The experiments of Bartlett (15) and Erlanger, Gesell, et al. (16), however point to a reduced vascular tone. In view of such results, the tendency of the present time is to favor the view that a reduction in peripheral resistance plays no part in the failure of the circulation in shock (cf. Henderson (17)).

With the direct demonstration in these experiments that a reduction of peripheral resistance occurs it is important to recall that the total resistance offered to the exit of blood from the arterial trunks is not entirely controlled *a*, by the state of arterial tone but may be modified; also *b*, by the caliber of the capillaries; *c*, by the "head on" pressure in the peripheral veins; *d*, by variations in extra vascular support; and *e*, by the viscosity of the blood. Furthermore, the total peripheral resistance is determined by the combined resistances in all the peripheral branches. It is therefore quite conceivable that the arterioles in the organs specifically subjected to trauma dilate and that a compensatory constriction occurs in other organs not so affected, in which case the total resistance to the aortic blood might still be reduced.

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THE MODE OF ACTION OF FOOD IN INCREASING OXIDATION

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Lavoisier, shortly after his discovery that oxygen supported combustion, showed that food, work and cold increased oxidation in the body. Rubner (1) showed that of the foodstuffs, meat ingestion increased oxidation most, fat next and sugar least. Investigations have also been carried out to determine the stimulating effect of the different amino acids and of the sugars on metabolism, and it has been found that the different amino acids as well as the different sugars vary greatly in this respect. Lusk (2) found, for example, that the ingestion of glycocoll and alanin greatly increased heat production, leucin and tyrosin increased it very little while glutaminic acid was without effect. Several theories have been advanced in attempts to explain how food increases oxidation in the body. For discussion and literature on the subject consult Lusk, *The Science of Nutrition*, 1917; Dakin, *Oxidations and Reductions in the Animal Body*, 1912; and Kastle, *The Oxidases*, Bulletin 59, 1910, Hygienic Laboratory, Washington, D. C.

We (3) have shown that when oxidation is increased, as for example, by increasing the amount of work, by thyroid feeding, by fighting, in the excitement stage of anaesthesia there occurs a corresponding increase in catalase, and that when oxidation is decreased, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, in deep narcosis, in "surgical shock," or rendered defective as in pancreatic diabetes, there occurs a corresponding decrease in catalase. From these results it was concluded that it is probable that catalase, an enzyme found in the tissues and possessing the property of liberating oxygen from hydrogen peroxide, is involved in the oxidative processes, possibly in the manner suggested by Bach and Chodat. The object of the present investigation was to determine if the ingestion of food increases the catalase of the tissues and, if so, how this is brought about. Dogs were used in the investigation. The experiments were begun by

depriving the animals of food for twenty-four hours. At the end of this time, 300 cc. of a peptic digest were introduced into the stomach of each animal by means of a stomach tube, and 100 grams of finely ground lean steak were given also. The digest was made by adding 50 grams of a commercial preparation of pepsin to 75 grams of finely ground lean beef in 300 cc. of 0.5 per cent hydrochloric acid solution. The mixture was permitted to stand in a thermostat at 40°C. for twenty-four hours. Previous to the introduction of the digest into the stomach of the animals at least two determinations were made of the catalase of the blood taken from the external jugular vein. After the introduction of the digest the determinations of the catalase of the blood were also made at intervals of thirty minutes. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. After the volume of gas thus collected in ten minutes had been reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken at a fixed rate of one hundred and eighty double shakes per minute during the determinations.

In figure 1, curve 1 was constructed from data obtained from a dog previous to and after the administration of 300 cc. of a peptic digest. The figures along the abscissa indicate time in minutes while the figures along the ordinate represent the amounts of catalase measured in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood. It will be seen that 0.5 cc. of the samples of blood taken previous to the introduction of the digest liberated 42 and 42 cc. of oxygen respectively; that thirty minutes after the digest was introduced, the blood liberated 48 cc. of oxygen; that sixty minutes later, it liberated 55 cc. of oxygen; after ninety minutes, 52 cc., and after one hundred and twenty minutes, 50 cc. It will be seen from this curve that the effect of the digest was to increase the catalase of the blood during the first hour by about 30 per cent, while during the second hour it was decreased. Curve 2 was constructed from data obtained in a similar manner as was that for curve 1, except this dog vomited the digest shortly after it was introduced. Curve 3 was obtained from a normal dog without the administration of anything. Curve 4 was obtained from a poorly fed dog. This dog was not at all particular about its food, eating everything that was given to him. Previous to and after the introduction of the digest, he gnawed on bones

continuously except at the times when the blood was taken from the external jugular. It will be seen that the catalase of the blood of this animal was increased by about 160 per cent one hour after the intro-

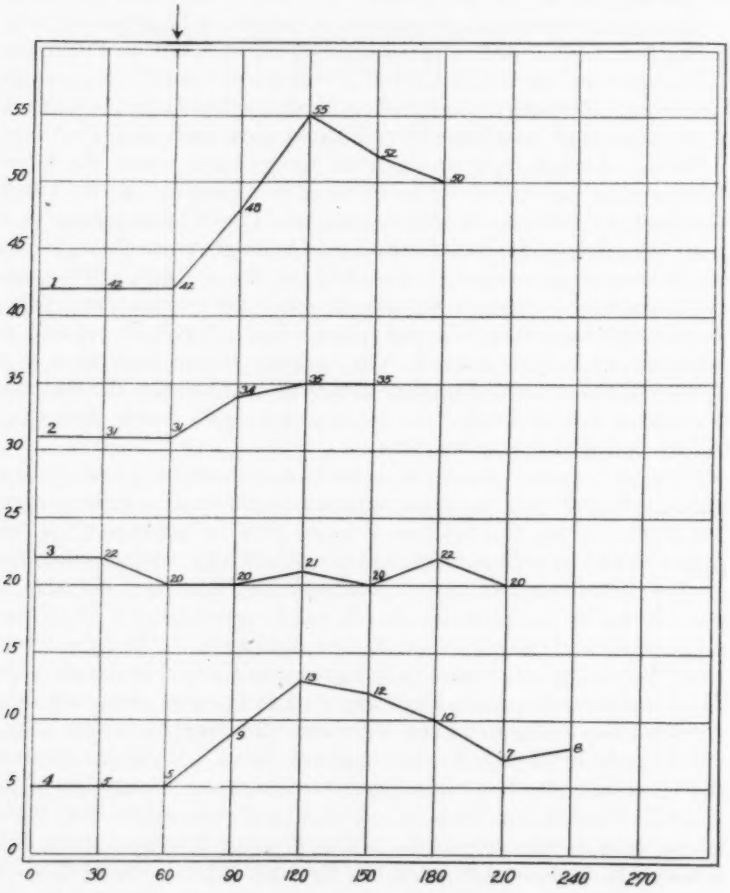

Fig. 1. Curves showing effect of food on the catalase content of the blood. The figures along the abscissa (0 to 270) indicate time in minutes, while the figures along the ordinate (0 to 55) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

duction of the digest, and that it had returned practically to the normal two and a half hours later. The low catalase of the blood of this dog is attributed to poor nutrition. These results show that food increases the catalase of the blood and hence of the tissues, parallel with the increase produced in oxidation.

The second part of this paper is concerned with determining how the ingestion of food produces an increase in catalase with resulting increase in oxidation. The digests used in the preceding experiments were tested for catalase and found to be negative, so the increase in the catalase of the blood could not have been due to catalase contained in the absorbed material. Furthermore, the contents of the stomach were also found to contain very little or no catalase, hence the increase in the catalase of the blood produced by the food must have been due to the stimulation of some organ or organs to an increased output of this enzyme. We had already found that the introduction of alcohol into the stomach of an animal greatly increased the catalase of the blood, and for this reason alcohol was the stimulant used in our attempts to determine what organ or organs are responsible for the output of catalase into the blood.

All the curves in figure 2 except curves 6 and 8 were constructed from data obtained from dogs previous to and after the administration of alcohol. Curve 1 was constructed from data obtained from three dogs whose livers had been cut out of the circulation by means of Eck fistulae and by the ligation of the hepatic arteries; curve 2, after the extirpation of the pancreas and spleen of two dogs; curve 3, from a dog into whose stomach 150 cc. of 45 per cent ethyl alcohol had been introduced through the walls of the stomach by means of a hypodermic needle after tying a ligature around the pyloric sphincter, thus preventing the passage of the alcohol into the intestines; curve 4, from a normal dog into whose stomach alcohol was introduced by means of a stomach tube; curve 5, after the extirpation of the pancreas; curve 6, from a normal animal without the administration of anything; curve 7, from two dogs into whose intestines 150 cc. of alcohol had been introduced through the walls of the intestines by means of a hypodermic needle after a ligature had been tied around the pyloric sphincter to prevent the passage of the alcohol into the stomach; curve 8, from a dog into whose jugular vein secretin, prepared according to the method of Starling, had been injected. In curve 1 it will be seen that after cutting the liver out of the circulation, the introduction of 150 cc. of 45 per cent ethyl alcohol into the stomachs of the animals increased the

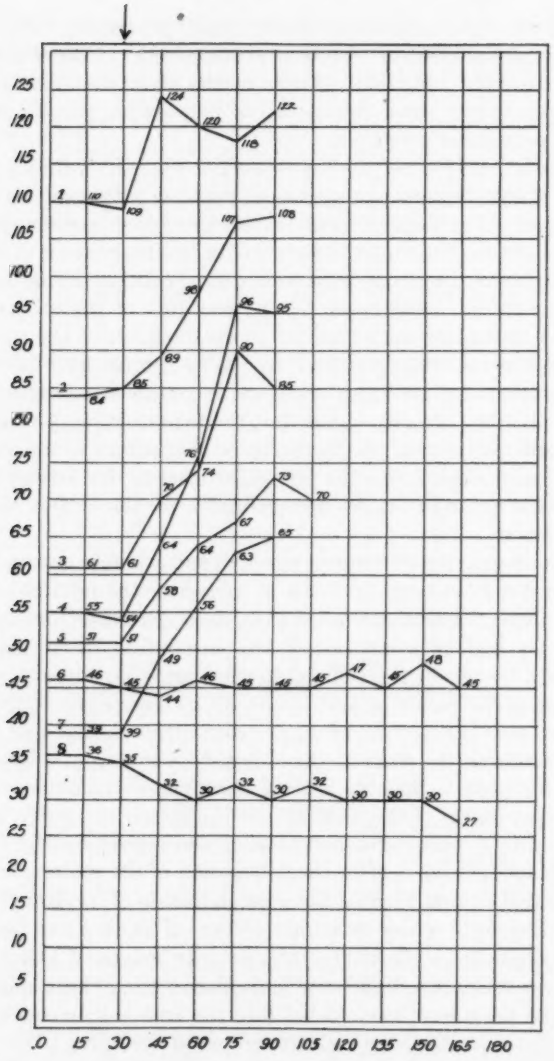

Fig. 2. Curves, except 6 and 8, showing effect of alcohol on the catalase content of the blood. The figures along the abscissa (0 to 180) indicate time in minutes, while the figures along the ordinate (0 to 125) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

catalase of the blood by about 13 per cent; while the introduction of a similar amount of alcohol into the stomach of a normal animal increased the catalase of the blood by about 80 per cent, as shown in curve 4, hence cutting the liver out of the circulation decreased the stimulating effect of alcohol by about 80 per cent. In curve 2 it will be seen that after the extirpation of the pancreas and spleen, the introduction of alcohol into the stomach increased the catalase of the blood by about 30 per cent, showing a decrease in the stimulating effect of alcohol by about 62 per cent from the normal. In curve 3 the introduction of alcohol into the stomach when the stomach was tied off increased the catalase of the blood by about 50 per cent; in curve 5 after the removal of the pancreas, alcohol increased the catalase by about 40 per cent; in curve 6 without the administration of anything, the catalase of the blood remained practically unchanged; in curve 7 after the administration of 150 cc. of alcohol into the intestines by means of a hypodermic needle when the pyloric sphincter was tied off, so that no alcohol could escape into the stomach, the catalase was increased by about 70 per cent; in curve 8 when secretin was injected into the external jugular vein of the dog, there was a small decrease in the catalase of the blood.

By comparing the data from which these different curves were constructed, it will be seen that the introduction of alcohol into the stomach of the normal animal greatly increased the catalase of the blood, while the introduction of a similar amount of alcohol into the stomach of animals whose livers had been shut off from the circulation, increased the catalase of the blood somewhat but not very extensively. This observation is interpreted to mean that the liver is one of the organs and probably the principal organ, stimulated by the alcohol to an increased output of catalase, thus producing the increase in the catalase of the blood. It will also be seen that the extirpation of the pancreas and spleen decreased the output of catalase into the blood after the introduction of alcohol into the stomach, showing that these organs, too, probably take part in the production of catalase after the administration of alcohol. That catalase is given off from the gastric and intestinal glands after the administration of alcohol is shown by the fact that the blood of the portal vein is the first to show an increase in catalase after the administration of alcohol.

From these observations it may be concluded that alcohol increases the catalase of the blood by stimulating the pancreas, the spleen, the gastric and intestinal glands and particularly the liver to an increased output of this enzyme. It is permissible, perhaps, to assume that food

like alcohol stimulates the production of catalase in the organs mentioned and in this way causes an increase in oxidations.

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THRESHOLD VALUES IN THE SPINAL FROG

I. Comparison of the Flexion Reflex and the Nerve-Muscle Response

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The calibration of the inductorium by Martin (1) has made possible not only the accurate measure of the physiological intensity of induction shocks, but a statement of the value of these stimuli in units which can be used as standards of comparison or which can be duplicated in any laboratory. The completion of the calibration is so recent that only a small amount of data has been obtained regarding the irritability of various tissues. Martin (2) has applied his method chiefly to the determination of sensory thresholds under various conditions. Porter (3) has studied the variations in the irritability of the reflex arc in the spinal cat under normal conditions, in asphyxia and under the influence of strychnine. No observations taken by the Martin method have been reported on the thresholds of electrical stimulation for reflexes in cold-blooded animals. Such data would make possible a comparison of the irritability of various tissues in warm- and cold-blooded animals under similar conditions. It is of biological interest to know whether the threshold for the flexion reflex is of the same order of magnitude in the frog and in the cat.

The threshold values given in this paper are from a series of experiments which were preliminary to an investigation of the effect of changes of temperature on the synapse. They support the view that the synapse is a point of resistance in the reflex arc.

PROCEDURE

The preparation used was the spinal frog (Rana pipiens) made by destroying the brain in the ordinary manner. The shock of the operation disappeared entirely in about fifteen minutes, when the animal took the characteristic posture of a spinal frog and reflexes could be

easily elicited. During the period before recovery the frog was prepared for the measurement of the threshold of the flexion reflex in the left hind leg, and the right hind leg was prepared for the threshold of a nerve-muscle preparation giving extension of the foot. A section of the left sciatic nerve about 3 cm. long was freed from the surrounding tissue and a silk ligature placed just below the point of branching above the knee. Distal to the ligature the nerve was destroyed. The nerve could then be lifted over the point of a Lucas (4) fluid electrode. The right sciatic was exposed and a ligature placed high in the thigh. Central to that point the nerve was destroyed. This nerve could also be slipped into the groove of a Lucas electrode.

Before the measurement of the thresholds the frog was suspended by its jaws from a femur clamp attached to a stand. The points of the electrodes were slipped under the nerves, the electrodes themselves being supported by clamps from the same stand. The whole set-up could thus be moved without disturbing the adjustment of the electrodes. The frog and electrodes were placed in a glass jar of cold-blooded Ringer's solution or in 0.7 per cent sodium chloride at room temperature.

An example of an experiment in which both Z and β were determined is given below. In this case Z was obtained with three different amounts of secondary resistance for greater accuracy in calculating β.

Experiment 4. The frog was prepared as described above for the determination of the reflex threshold. The left sciatic nerve was placed in the slot of the electrode. The primary current was made and broken by means of a Martin key (5) which short-circuited the make shocks. The resistance in the primary circuit was adjusted so that 0.1 ampere passed through the primary coil. The secondary coil was pushed out to the end of the scale and while the current was repeatedly made and broken the secondary was gradually moved toward the primary. A point was reached where the slightest perceptible flexion of the left leg occurred in response to a break shock, which marked the threshold for the reflex. The secondary position was 21.5 cm. A calibration table of the values for $\frac{M}{L}$ for the inductorium used gave 78.7 for this position of the secondary. Since $Z = \frac{MI}{L}$, for this determination Z becomes 78.7×0.1, or 7.87. Then 10,000 ohms additional resistance were put in the secondary circuit by withdrawing plugs from a resistance box. Again the threshold was determined and the Z calculated was 19.7. With 20,000 ohms additional the third value of Z was 34.3.

The resistance of the nerve was determined by the Kohlrausch (6) method and found to be 2000 ohms. The secondary coil itself had a resistance of 850 ohms. With the values of Z and the resistances two values of β were calculated according

to the formula developed by Wilbur (7). The values obtained were 4.4 and 4.2. For this experiment $\beta = 4.3$, $Z = 7.9$, $\frac{\beta}{Z} = 0.55$.

PRELIMINARY EXPERIMENTS

Before making a series of threshold determinations of the ipsilateral flexion obtained on stimulating the central end of the sciatic nerve, it was necessary to determine with certainty that the response was a reflex. Lucas (8) has reported that with the fluid electrode a spread of current to tissue 5 mm. distant could only be obtained by increasing the strength of the current eighty times that of the threshold stimulus. In all of the experiments reported here the neighboring tissue, with the exception of the nerve itself, was from 5 mm. to 10 mm. distant from the slot in the electrode. Spread from threshold strengths would hardly be expected. The response obtained was always clear cut flexion. With direct stimulation by spread of current to nerve endings or muscle fibers an indefinite response would be expected since both flexors and extensors were equidistant from the point of the electrode. When the dorsal and ventral roots were cut or the cord pithed, the response obtained with increased strength of stimulus was always a combination of the contractions of both sets of muscles.

Direct evidence as to the nature of the response was obtained by determining the threshold of the response before and after cutting the spinal roots or pithing the cord, and also by comparing the results obtained with the thresholds of the nerve-muscle preparation in the opposite leg subjected to the same conditions. The results showed that in order to obtain a response after pithing the cord, the stimulus had to be increased from forty to one hundred times, while of course no increase was necessary to obtain the nerve-muscle response in the opposite leg. One seems justified in concluding that the clear cut flexion obtained at the lower value was a reflex. The procedure of destroying the cord at the end of the experiment was carried out as a matter of routine in each experiment. A few of the results are given in table 1.

RESULTS

The values reported in table 2, are the threshold stimuli in Z units and in β units for the flexion reflex and for the nerve-muscle extensor preparations in spinal frogs prepared as described above. The determinations have been made at least fifteen minutes after pithing and usually before an hour had elapsed.

The most important results are those of β for the reflex and for the nerve-muscle preparation. The thirty-four values of β for the reflex obtained from different animals average 6.9, and the thirty for the nerve-muscle preparation give an average of 4.7. The higher threshold for the reflex is in accordance with other wellknown characteristics of the reflex arc summarized by Sherrington (9). The average value of Z for the reflex is 11.7, while that for the nerve-muscle preparation is 4.7.

TABLE 1

Results of experiments to show that the response obtained from the stimulation of the central end of the sciatic is a reflex and is not due to current spread

DATE	NO.		REFLEX THRESHOLD Z UNITS	NERVE-MUSCLE THRESHOLD Z UNITS
11/30/15	4	Before pithing cord	9.4	
		After pithing cord	1176.0	
12/11/15	12	Before pithing cord	8.6	4.4
		After pithing cord	236.5	4.3
1/19/16	20	Before pithing cord	17.1	
		After pithing cord	1248.0	
12/ 4/16	28	Before pithing cord	34.3	6.2
		After pithing cord	266.4	5.9
12/ 5/16	29	Before pithing cord	10.5	3.4
		After pithing cord	675.0	3.5
12/19/16	39	Before pithing cord	7.7	7.2
		After pithing cord	618.0	9.7

The calculation of β in many experimental procedures may become too tedious and results stated in Z units may be sufficient for the purposes of the experiment. It is therefore of practical importance to know the ratio of β to Z for the tissue used, if the results are to be stated only in Z units. In the case of the reflex this value is found to average 0.59. For the nerve-muscle preparation it is 0.56. For the reflex the greatest deviation from the average ratio is 0.18 or 32.8 per cent. The average deviation is 0.09 or 16 per cent. In the case of the nerve-muscle preparation, the greatest deviation is 0.31 or 55 per cent. The average deviation is 0.07 or 13 per cent.

It will be noted in table 2 that a few of the values for Z and β deviate

TABLE 2

DATE 1915–1916	INTERVAL AFTER PITHING BRAIN	REFLEX FLEXION OF HIND LEG—SCIATIC			NERVE-MUSCLE EXTENSION OF FOOT—SCIATIC		
		Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$
	h. m.						
11/24/15*	1.00	2.2	1.6	0.72			
11/30/15	†	8.5	5.9	0.69			
12/ 2/15	0.15	5.8	3.1	0.44	3.5	1.5	0.25
12/ 5/15	0.30	11.0	5.0	0.45	5.5	3.4	0.62
12/ 5/15	0.10	14.6	10.6	0.73			
12/ 6/15	0.10	2.9	1.2	0.41	1.9	1.1	0.58
12/ 7/15	0.30	4.7	2.3	0.49	3.0	1.7	0.57
12/10/15	0.20	8.0	4.5	0.53	4.7	2.7	0.57
12/11/15	0.10	4.9	3.6	0.73	3.4	1.8	0.53
12/14/15	0.20	12.2	6.4	0.52	4.5	2.6	0.58
1/ 5/16‡	0.15	9.5	5.7	0.60			
1/ 5/16‡	0.25	7.5	4.1	0.55	2.3	1.5	0.65
1/ 5/16‡	0.15	8.6	4.2	0.49	2.2	1.2	0.56
1/ 6/16	0.15	27.0	17.8	0.66	4.2	2.5	0.59
1/17/16	0.54	7.2	3.5	0.48	2.0		
1/19/16	0.35	17.5			8.4		
1/19/16	0.32	9.2			4.4		
1/20/16	0.35	24.8			6.6		
1/20/16	0.20	8.3	4.6	0.55			
1/25/16	1.09	7.4					
1/26/16	0.35	37.0	26.4	0.70	4.9	2.2	0.45
1/26/16	0.20	44.4	27.0	0.61	11.2	7.3	0.65
1/26/16	0.15	20.4	11.6	0.57	5.3	3.4	0.64
12/ 4/16	0.20	25.9	12.9	0.50	6.6	4.0	0.61
12/ 6/16	0.20	10.5	5.8	0.55	3.0	1.0	0.33
12/ 6/16	0.15	7.2	3.5	0.49	3.4	2.1	0.62
12/ 7/16	0.22	9.0	5.2	0.57	7.8	4.7	0.60
12/ 7/16	1.10	15.8	9.4	0.59	9.9	5.2	0.53
12/ 8/16	0.47	6.1	3.1	0.51			
12/14/16	0.45	6.6	4.4	0.67	2.4	1.5	0.62
12/16/16	1.05	11.9	9.1	0.76	8.8	6.7	0.76
12/16/16	1.20	3.0	2.0	0.67			
12/19/16	0.55	5.4	2.4	0.44	5.1	3.0	0.59
12/19/16	0.15	4.4	3.3	0.75			
Average 1: All experiments		11.7	6.9	0.59	4.7	2.7	0.56
Average 2: Omitting all 100 per cent from Average 1.....		8.6	4.6	0.54	4.3	2.5	0.56

* Platinum points; electrodes in air.

† Average of five observations during one hour.

‡ Central end of IX root (abdomen open).

more than 100 per cent from the average. The frogs which gave these extremely high thresholds were always confined to shipments of frogs which had been delayed in transit from the west and had suffered exposure to cold. It may be pointed out, however, that the ratio of β to Z was very little altered in these cases. Here the greatest variation from the average is only 0.11 or about 19 per cent. It seems justifiable, therefore, to omit the values obtained from these frogs in making corrected averages.

The revised averages for Z and β for the flexion reflex are 8.6 and 4.7 respectively. For the nerve-muscle preparation, in which wide variations were very few, the corrected values of Z and β are 4.3 and 2.5. The corrections have altered only slightly the ratio of β to Z, which becomes 0.54 for the reflex and remains 0.56 for the nerve-muscle preparation. Martin (10) gives 8.1 as the average β for 18 observations on the frog's gastrocnemius stimulated directly with platinum points. He finds the ratio of β to Z to be 0.49, and the greatest deviation about 33 per cent. The average variation was 15 per cent. Porter (11) reports the average threshold stimulus for the flexion reflex in the spinal cat (seventeen determinations) to be 2.7 β units. An extensor nerve-muscle preparation at the wrist he found to have a threshold of 1.4 β units. The average Z units for threshold stimuli for the reflex (sixty-six determinations) was 5.2, and for the nerve-muscle preparation (fifty-two determinations) it was 2.3. He found the average ratio of β to Z to be 0.57, calculated from the combined results of reflex and nerve-muscle preparation. He does not state these values separately.

In comparing the results of Porter with those presented in this paper, one should consider that the thresholds on the spinal cat were taken at 38°C. while those on the spinal frog were taken at room temperature, from 15°C. to 20°C. That this difference of threshold might well be accounted for on a temperature basis alone will be shown in a subsequent paper.

It is interesting to compare the values reported here and those of Martin and Porter, with the thresholds for sympathetic responses in the cat reported by Mendenhall (12). He found the thresholds for contraction of the pupil through cervical sympathetic stimulation to be 5.7 Z units and 3.3 β units. The threshold for retraction of the nictitating membrane stimulated in the same manner was 6.3 Z units and 3.7 β units. For vasoconstriction in the nasal vessels, elicited through the cervical sympathetic, he found the threshold to be 7.9 Z units or 4.6 β units, and the ratio of β to Z in these observations was 0.58.

The values 5.7 Z, 6.3 Z, 7.9 Z obtained by Mendenhall for autonomic thresholds, 5.2 Z for reflex threshold in the cat reported by Porter, and 8.6 Z for reflex thresholds in the frog reported in this paper, are suggestive when compared with the thresholds for nerve-muscle preparations, as 2.3 Z by Porter for the cat, and 4.3 Z in this paper for the frog. It is usually accepted as a fact that each synapse increases the resistance to the passage of an impulse, although up to the present the only quantitative evidence has been that presented by Sherrington and Sowton (13) and Porter (14). In the autonomic and reflex paths, from which the above values were obtained, at least one synapse is known to exist.

The results of this investigation, showing a difference of 4.3 Z units, or 2.1 β units between reflex and nerve-muscle preparation, bear out Porter's observations which suggest strongly that a certain amount of resistance lies in the synapse.

SUMMARY

1. The fluid electrodes of Lucas have been used to determine the threshold stimuli for the flexion reflex and for a nerve-muscle preparation in the spinal frog. The values are reported in the Z and the β units of Martin.

2. The average threshold stimulus for twenty-five determinations of the flexion reflex in the spinal frog is 4.6 β units. For the nerve-muscle preparation the average threshold stimulus for twenty determinations is 2.5 β units.

3. The average threshold value in Z units for twenty-eight determinations of the flexion reflex is 8.6; and for twenty-three determinations of the nerve-muscle preparation it is 4.3.

4. The average ratio of β to Z for the reflex (thirty determinations) is 0.59. The average deviation is 0.09 or 16 per cent. The average ratio of β to Z for the nerve-muscle preparation (twenty-one determinations) is 0.56. The average deviation is 0.07 or 13 per cent.

5. The values of threshold stimuli for the flexion reflex and for the nerve-muscle preparation support the view that the synapse is a point of resistance in the conduction path of a reflex arc.

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THRESHOLD VALUES IN THE SPINAL FROG

II. Variations with Change of Temperature

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The Martin method makes possible an exact study of the effects of various conditions on reflex thresholds. Since 1860, when Kunde (1) came to the conclusion that reflexes were depressed by cooling, the effects of changes of temperature on reflex irritability have been studied by many investigators. Statements which are quite conflicting are found in the literature. Recently (1916) Van Leeuwen and Van der Made (2) have reported that the optimum temperature for reflex irritability in the winter frog lies at about 5°C. and 2°C. higher for the summer frog. They find in some cases a second optimum around 20°C.

That there should be a disagreement about the effect of cold on reflexes might at first seem strange, when it is well known that cooling depresses the activity of protoplasm (3). But the explanation of the varied results will probably be found in the means used in determining the reflex irritability. The most common method has been that of Türch, in which the foot of the frog is dipped in acid and the time before its withdrawal taken as an index to the reflex activity. Since the introduction of induction coils into physiological work faradic stimuli have been applied directly to the skin of the leg or foot. In both of these procedures the sensory end-organs are involved and summation of stimuli is a chief factor. It is obvious that such methods cannot give so consistent results as one in which single break shocks of known intensity are applied directly to the afferent nerve and the threshold thus determined at various degrees of temperature.

But of perhaps greater physiological interest is the effect of change of temperature on the reflex threshold when it is compared with the threshold of a nerve-muscle preparation subjected to identical conditions. Sherrington (4) has summarized the characteristic differences between conduction in nerve trunks and in reflex arcs. Among other differences,

conduction in the reflex arc exhibits a greater variation in threshold value of a stimulus, a greater fatigability, much greater dependence on blood circulation and oxygen and a greater susceptibility to drugs. One might expect that cooling would also have a greater effect on reflex arc conduction, in which synapse and cell body are concerned, than in nerve trunk conduction.

Many observers working in this field have failed to distinguish between a change of temperature as a condition and a change of temperature as a stimulus. This is especially true of the early investigators. Heinzman (5), Goltz (6), Foster (7) and Sedgwick (8) were at one time concerned with the question of the effect of gradual application of heat on reflex excitability, as when a decapitated frog is placed in a water bath and heated.

Githens (9) has repeated and extended Kunde's experiments on the action of strychnine at various temperatures, finding that a given dose causes a longer period of spasms at 5°C. than at 31°C. He concludes that 5°C. is the optimum temperature for this drug. It seems curious, however, that this greater duration of activity could be due to the irritability of the cord determined by the low temperature. The possibility that the drug was eliminated more slowly at low temperatures was not considered.

The objects of this paper are to present the results of a series of experiments in which the effects of changes of temperature on reflex irritability and on nerve-muscle irritability are compared, and to consider these results in the light of the "all or nothing" principle.

PROCEDURE

A spinal frog was prepared in the ordinary manner and both sciatic nerves exposed sufficiently to slip easily into the groove of the Lucas fluid electrode (10). The left sciatic was used to evoke reflex flexion in the same leg, the nerve being destroyed at the knee. The right sciatic was destroyed high in the thigh and the lower leg used for nerve-muscle extension of the foot. The two responses used differed only in that the reflex arc contained afferent nerve fibers, cell body and synapse in the conducting path, in excess of the efferent fibers, motor endings and muscle fibers which were involved in both responses alike. Sensory end-organs were excluded by stimulating the afferent trunk directly. Temporal summation of inadequate stimuli was avoided by using single break shocks given at sufficiently long intervals. The

time resistance was taken at the end of the experiment by the Kohlrausch method with the electrodes in place.

The frog was suspended by its jaws from a femur clamp attached to a stand. The points of the two Lucas electrodes were slipped under the nerves, the electrodes being supported by clamps from the same stand. The whole set-up could be transferred from one jar of Ringer to another of different temperature without disturbing the adjustment of the electrodes. The actual temperature of the frog was not taken as a matter of routine. A few experiments showed that the temperature of the frog as measured by a thermometer inserted through the oesophagus into the stomach became the same as that of the surrounding fluid in seven or eight minutes. In all cases sufficient time was allowed after changing the temperature for constant threshold responses to be obtained.

In order to exclude the possibility that a rise of threshold as the experiment proceeded might obscure temperature effects, the temperature of the animals was changed, in different experiments and sometimes in the same experiment, in both directions, as from low temperature to high, from high to low and then to high temperature again. These procedures eliminated time as a factor in the results. When the experiment was started at a low temperature the pithed frog was kept packed in ice from half to three-quarters of an hour previous to the first determination. Although the threshold values in Z units are sufficient for these experiments, for completeness the data necessary for calculating β were sometimes obtained; i.e., three values of Z at different secondary resistances for greater accuracy, and the tissue resistances.

PRELIMINARY EXPERIMENTS

Before studying the effects of changed conditions on the responses involved it was necessary to find the variations which might be expected under the conditions of the experiment at a given temperature. Sedgwick (11) has suggested that a suspended frog loses its reflex irritability after thirty or forty minutes especially if warmed. Such an effect as this would obscure the effects of temperatures and tend to raise the reflex thresholds as the experiment proceeded. Several experiments were performed, therefore, to test the constancy of the reflex response.

In experiment 4 the fluid electrodes were used and the constancy of the reflex followed for fifty-three minutes, a much longer period than the duration of any of the subsequent experiments on the effects of tem-

perature changes. Recently Porter (12) has shown that the flexion reflex in the spinal cat remains comparatively constant for an hour or more. The accompanying protocol and figure 1 show that within an hour the reflex thresholds are fairly constant at a given temperature.

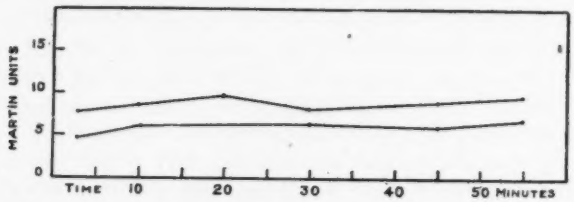

Fig. 1. Experiment 4, November 30, 1915. Variations in the threshold of the flexion reflex for a period of fifty-three minutes, plotted in Z and β units. Ordinate represents Martin units; abscissa, time in minutes.

Protocol of experiment 4, November 30, 1915

2.20 p.m. Brain pithed through foramen magnum.
2.25 p.m. Left sciatic nerve exposed.
2.30 p.m. Lucas electrodes applied to central end of sciatic.
2.40 p.m. Preparation immersed in cold-blooded Ringer solution at 20°C.
Primary current 0.1 ampere.

TIME	TISSUE RESISTANCE AND COIL			10,000 OHMS ADDED			20,000 OHMS ADDED			0 OHMS
	Coil	$\frac{M}{L}$	Z	Coil	$\frac{M}{L}$	Z	Coil	$\frac{M}{L}$	Z	β
2.43	21.5	78.0	7.8	17.3	197	19.7	15.5	343	34.3	4.3
2.50	21.3	81.6	8.2	17.4	190	19.0	15.5	343	34.3	6.0
3.00	20.5	95.0	9.5							
3.10	21.4	80.4	8.0	17.1	209	20.0	15.7	319	31.9	6.4
3.25	21.0	86.0	8.6	17.0	216	21.6	15.6	331	33.1	6.1
3.40	20.4	97.0	9.7	16.9	222	22.2	15.5	343	34.3	7.2
Average...			8.6							6.0

The greatest deviation from the average for Z in experiment 4 was 1.1, or 12.8 per cent, while the average variation was only 0.6, or 7 per cent. For β the maximum variation from the mean was 1.7, or 28 per cent, while the average variation was 0.60, or 10 per cent. The ratio of β to Z was 0.69.

RESULTS

The figures presented in table 1 show clearly two important points. First, cooling increases the thresholds for both reflex and nerve-muscle response, while warming produces the reverse effect. Second, the reflex threshold is affected to a much greater degree than the nerve-muscle threshold by changes of temperature.

The first conclusion is to be expected from the general effect of lowering the temperature on biological processes, and the values obtained in these experiments without exception support this law. A few figures will serve to illustrate this point. In the experiment of De-

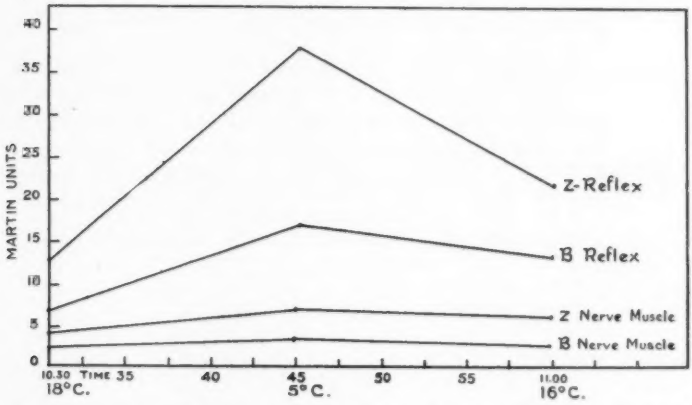

Fig. 2. Experiment 15, December 14, 1915. Reflex and nerve-muscle thresholds taken at 18°C., 5°C., and 16°C.

cember 11, 1915, a fall of temperature from 18°C. to 6°C. was accompanied by a rise of the reflex threshold from 4.9 Z to 10.7 Z, 3.5 β to 8.6 β, or a rise of about 130 per cent. The experiment of December 7, 1916, showed an increase of the reflex threshold from 10.5 Z to 24.7 Z or 5.2 β to 16.3 β caused by a fall of temperature from 20°C. to 6°C. The change here was about 170 per cent. The effect of a rise in temperature was shown strikingly in the experiment of January 17, 1916, when a change from 5°C. to 30°C. caused a lowering of the reflex threshold from 25.4 Z to 2.3 Z or 92 per cent. The nerve-muscle threshold in the same experiment fell from 3.0 Z to 1.5 Z or 50 per cent. A rise in the nerve-muscle threshold was shown in the experiment of December 14, 1916,

TABLE 1

Results of eleven experiments to compare the effect of change of temperature on reflex and nerve-muscle thresholds

DATE	TIME	TEMPERATURE	REFLEX				NERVE-MUSCLE			
			Z	Change	β	Change	Z	Change	β	Change
		deg. C.								
12/11/15	11.10	18	4.9		3.5		3.4		1.8	
	11.35	6	10.7	5.8	8.6	5.3	4.5	1.1	2.4	0.6
	12.00	16	8.6	2.1	6.9	1.7	4.4	0.1	2.9	0.5
12/14/15	10.30	18	12.0		6.4		4.4		2.5	
	10.45	5	38.0	26.0	17.1	10.7	7.2	2.8	3.6	1.1
	11.00	16	22.5	15.5	13.7	3.4	6.5	0.7	3.4	0.2
1/ 5/16	2.25	20	7.5		4.1		2.3		1.5	
	2.37	6	14.4	6.9	7.7	3.6	2.7	0.4	1.6	0.1
	2.55	19	10.3	4.1	5.0	2.7	3.1	0.4	1.9	0.3
1/17/16	3.35	5	25.4		18.0		3.0			
	3.44	16	7.8	17.6	3.8	14.2	2.0	1.0		
	3.49	16	7.2	0.6	3.5	0.3	1.8	0.2		
	3.57	30	2.3	4.9			1.5	0.3		
1/19/16	5.04	5	24.0				11.2			
	5.10	17	17.5	6.5			8.4	2.8		
1/19/16	2.25	5	24.0				9.9			
	2.35	15	15.6	8.4			5.9	4.0		
	2.45	24	9.2	6.4			4.4	1.5		
1/20/16	2.25	5	58.8				7.9			
	2.30	16	30.2	28.6			6.5	1.4		
	2.40	25	24.8	7.4			6.6	0.1		
	2.48	5	58.8	34.0			8.1	1.5		
	2.59	30	22.2	36.6			6.5	1.6		
1/25/16	4.15	16	17.1				16.6			
	4.32	8	20.4	3.3			17.1	0.5		
	4.39	32	7.4	13.0			14.4	2.7		
1/26/16	10.10	18	49.7				5.2			
	10.19	5	62.4	12.7			8.5	3.3		
	10.30	18	40.8	21.6			6.7	1.8		
	10.43	30	35.5	5.3			6.6	0.1		

TABLE 1—Continued

DATE	TIME	TEMPERATURE	REFLEX				NERVE MUSCLE			
			Z	Change	β	Change	Z	Change	β	Change
		deg. C.								
12/ 7/16	9.52	20	10.5		5.2		7.8		4.7	
	10.08	6	24.7	14.2	16.3	11.1	11.4	3.6	6.2	1.5
	10.22	20	18.5	6.2	9.8	6.5	10.9	0.5	6.5	0.3
12/ 7/16	2.44	4	18.5				10.5			
	2.53	18	12.5	6.0			9.9	0.6		
	3.07	7	26.9	14.4			11.2	1.3		
	3.18	18	15.3	11.6			10.7	0.5		

when a fall of temperature from 18°C. to 5°C. caused a change from 4.4 Z to 7.2 Z, about 60 per cent.

These figures and the others presented in table 1 seem to show that not only is the nerve-muscle irritability depressed on cooling, but, for the range of temperature between 30°C. and 5°C., cooling depresses reflex irritability.

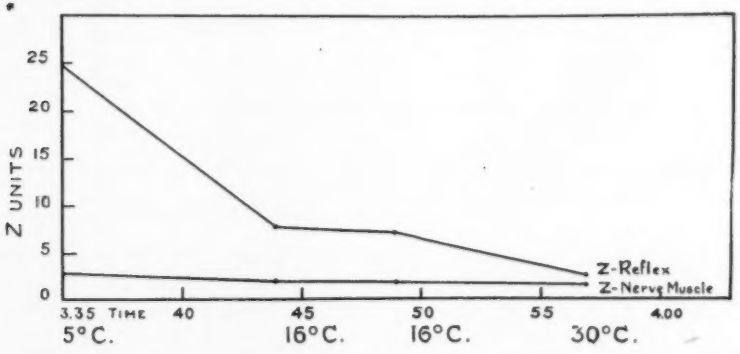

Fig. 3. Experiment 19, January 17, 1916. Reflex and nerve-muscle thresholds taken at 5°C., 16°C., 16°C. and 18°C. The frog was packed in ice for thirty-five minutes previous to the first determination.

The second conclusion, that the reflex irritability is more markedly affected by changes of temperature is in accord with other differences between the reflex arc and the nerve-muscle preparation. All of the determinations, with a single exception, in the eleven experiments gave

evidence for this point. In the experiment of December 11, 1915, for example, a fall of temperature of 12°C. caused an increase in the reflex threshold of 130 per cent, while the nerve-muscle threshold increased only about 32 per cent. In the experiment of January 17, 1916, a rise of temperature of 25°C. caused a fall of the reflex threshold of 92 per cent, and the nerve-muscle threshold was lowered but 50 per cent. An examination of table 1 will show other ratios of similar magnitude.

The figures in table 2 give the changes in Z units for a change of 1°C., both for the reflex thresholds and the nerve-muscle thresholds in each

TABLE 2

Change in Z units per 1°C. change of temperature

DATE	REFLEX	NERVE-MUSCLE
12/11/15	0.480	0.010
12/14/15	2.000	0.215
1/ 5/16	0.500	0.019
1/17/16	0.920	0.060
1/19/16	0.540	0.235
1/19/16	0.780	0.290
1/20/16	1.700	0.065
1/20/16	1.700	0.075
1/25/16	0.540	0.011
1/26/16	1.000	0.076
12/ 7/16	0.900	0.148
12/ 7/16	1.000	0.099
Average.....	1.005	0.109

experiment. The average change per degree Centigrade for the reflex threshold is 1.005 Z. For the nerve-muscle threshold this value is 0.109. The average deviation from the mean for the reflex is 0.31 or 31 per cent. For the nerve-muscle preparation the average variation is 0.074 or 68 per cent.

When the average change per degree for the reflex is compared with that for the nerve-muscle, the ratio is 9 to 1. In other words, for the range of temperature in these experiments the reflex threshold is affected nine times more than the nerve-muscle threshold by changes in temperature.

DISCUSSION

Little is known of the relative irritability of afferent and efferent fibers under various conditions nor is the phenomenon of conduction through the synapse well understood. If the reflex arc is more affected by changes of temperature than the efferent nerve fiber and muscle, as these experiments indicate, at what point in the reflex path does this greater effect take place? One must look to the afferent nerve, the synapse or the nerve-cell body, since the motor nerves and effector organs are common to both preparations.

There is no evidence that cold as a condition affects the irritability or conductivity of afferent fibers more than it does these phenomena

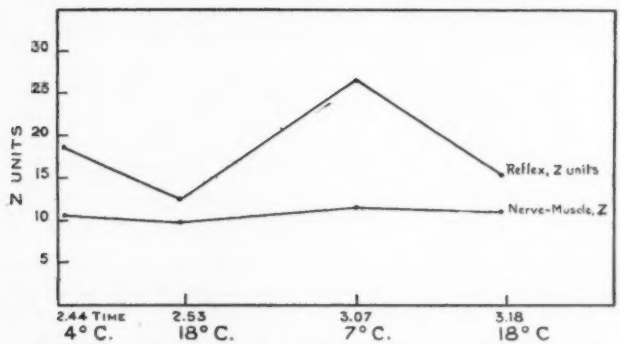

Fig. 4. Experiment 22, January 20, 1916. Reflex and nerve-muscle thresholds taken at 5°C., 16°C., 25°C., 5°C. and 30°C. The frog was packed in ice for fifteen minutes previous to the first determination.

in efferent fibers. Howell (13) states that motor fibers, however, are less easily stimulated by thermal applications than are sensory fibers, and suggests that this would seem to indicate some difference in structure or irritability between them. But until we have more direct experimental evidence that irritability and conductivity in afferent fibers are more susceptible to changes of temperature, it cannot be held that the afferent fibers would respond differently from the efferent fibers, either at low or high temperatures.

To the nerve-cell body is usually attributed the function of nutrition. Bethe (14) pictures the nerve fibrils passing directly through the synapse and cell body from one neurone to another. Whether such phenomena as "after-discharge," greater susceptibility to lack of oxygen, etc., are

due to the cell body, it is difficult to determine. Sherrington (15) rather favors the synapse as the point most easily affected by modifying conditions. The work of other investigators all indicates more or less directly that the characteristic features of reflex arc conduction are not referable to the nerve-cell bodies of the neurones. Bethe (16) in working on motor nerve-cells of Carcinus, and Steinach (17) in his studies on the physiology of the nerve-cells of the dorsal-root ganglia, suggest that the nerve-cell is little concerned with conduction. Langley (18) in showing that nicotine has little effect when applied to the dorsal-root ganglia, where no synapses are present, brings evidence to support the view that the synapse is the vital point in conduction through the reflex arc. It is probably the existence of the synapse

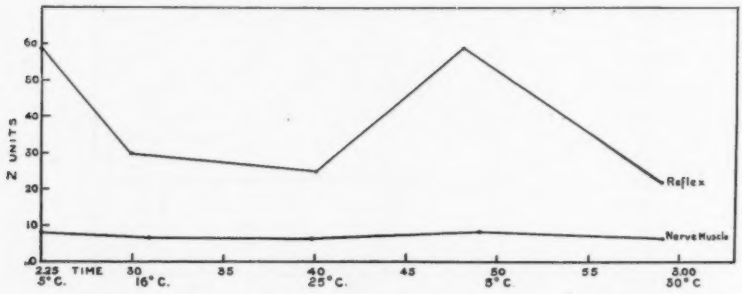

Fig. 5. Experiment 32, December 7, 1916. Reflex and nerve-muscle thresholds taken at 4°C., 18°C., 7°C., and 18°C. The frog was packed in ice for forty-five minutes previous to the first determination.

which makes the reflex arc so markedly susceptible to change of temperature.

How is a greater variation of threshold for the reflex arc than for the nerve-muscle preparation, when both are subjected to the same modifying conditions, to be reconciled with the "all or nothing" principle for nerve? In considering this question, it must be remembered that the threshold values obtained in the experiments reported here are an index of the number of nerve fibers stimulated to give a minimal response. A break shock of 2 Z, for example, stimulates all nerve fibers with thresholds lower than this value, and if 2 Z is the threshold value for the response, enough fibers must be stimulated to give the smallest discernible contraction.

For simplicity, let us suppose that the threshold of the nerve-muscle preparation N–M, figure 6, is 1 Z, while that for the reflex arc R is 2 Z. If these two preparations are cooled the threshold of N–M will be raised to 2 Z. If the "all or nothing" principle holds true for nerve, as Adrian's work seems to indicate, we may explain this rise in threshold by assuming that certain fibers are more susceptible to cooling than others and lose their function. But 2 Z will be strong enough to stimulate fibers of higher threshold that were not affected by 1 Z, and consequently enough functioning fibers are brought into activity at the higher threshold to give a minimal response. As far as the fibers themselves are concerned, we should expect that those of the reflex arc would be

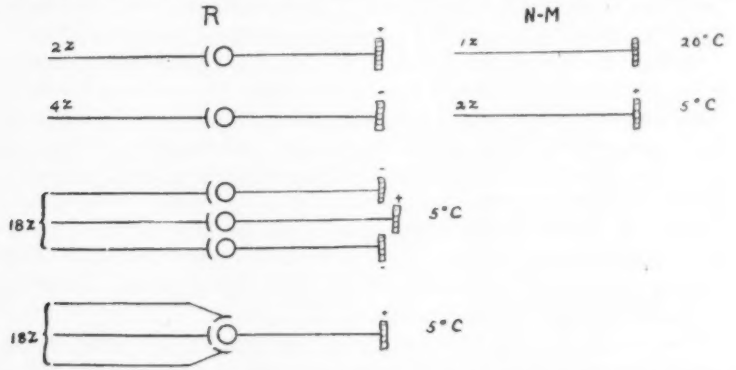

Fig. 6. In this diagram *R* represents the reflex arc with its synapse and nerve cell, *N-M* represents the nerve-muscle preparation. The + indicates a threshold response, the − indicates failure of the response at the stimulating values designated. Further explanation will be found in the text.

affected to the same degree by cooling, and a threshold of 2 Z would thus be raised to 4 Z. But a stimulating strength of 4 Z will not give a response. It is necessary to use a strength nine times as great as that for N–M under the new conditions, or 18 Z. It must be assumed that the synapses vary greatly, in themselves, in their susceptibility to cooling, so that although 2 Z at an ordinary temperature stimulated enough fibers with functioning synapses to give a minimal response, at a low temperature 18 Z must be used to stimulate enough fibers with synapses still functioning. There would be no necessity for any other view than that each nerve fiber, when stimulated by a shock sufficient to produce a local excitatory disturbance at the point of stimulation

great enough to discharge a propagated disturbance, responds with its maximum activity for the conditions.

Whether the actual propagated disturbance is smaller at low temperatures than at high is not known. Boruttau (19) has suggested that this is the case. Assuming this to be true, on cooling, enough afferent fibers carrying weakened impulses would have to converge on a single efferent fiber to produce a discharge through this path. In other words, there would be need for a sort of summation of stimuli through afferent fibers on a single efferent fiber. Very little is known about the passage of impulses from one neurone to another. The phenomenon of "after-discharge" suggests that some sort of summation may take place. And the greater number of afferent fibers than efferent fibers demands that more than one sensory fiber converge on a single motor neurone.

Adrian (20) has shown that anaesthetics affect the conduction of a nerve impulse by causing a progressive diminution in the intensity of the impulse until it finally ceases to exist. If cooling a nerve should cause a decrement in the size of the impulse as it passes away from its point of origin, this alone might account for the greater threshold necessary for the reflex response, because of the greater distance to the effector organs. A greater threshold stimulus would be necessary in order to affect a large enough group of fibers in which a certain proportion failed to conduct impulses to the muscle and in which enough fibers conducted the entire distance to produce a minimal response.

The results presented above may be interpreted according to the "all or nothing" principle by assuming that at low temperatures a greater threshold stimulus is necessary in order to include a sufficient number of functioning synapses, a certain number being entirely functionless at these temperatures. Or if it should be that the nerve impulse is much smaller at low temperatures, a greater number of fibers must be stimulated in order to conduct the weakened impulses to a smaller number of motor neurones, allowing summation and reinforcement to occur.

SUMMARY

1. The effects of changes of temperature on reflex arc and nerve-muscle preparation in the same animal are compared in spinal frogs. Changes of temperature were obtained, ranging from 4°C. to 30°C., by immersing the animal suspended by its jaws in normal saline or Ringer's solutions of various temperatures. The Lucas fluid electrodes were applied directly to the nerve-trunks.

2. Cooling depresses reflex irritability and nerve-muscle irritability, as indicated by a rise of the threshold strength of stimulus. Warming increases the irritability of both, as shown by a lowering of the threshold strength of stimulus.

3. Changes of temperature affect the reflex arc more than the nerve-muscle preparation. The average change per degree for the reflex is 1.01 Z units; and for the nerve-muscle, 0.11 Z units. The ratio is about 9 to 1.

4. The greater effect of cooling on the reflex arc is in accord with other differences between conduction in the reflex arc and conduction where no synapse is involved; and the results presented here suggest that the place of incidence of this greater effect is at the synapse.

5. The conclusions reached are reconciled with the "all or nothing" principle for nerve.

I wish to acknowledge my indebtedness to Dr. E. G. Martin, who gave me instruction in the use of his method, and to Dr. Alexander Forbes for his many suggestions.

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THE EFFECTS OF ADRENIN ON THE URINE FLOW OF ANESTHETIZED AND UNANESTHETIZED DOGS

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The existing mass of data concerning the physiologic and pharmacologic action of adrenin on the various functions of the body is, with but few exceptions, complicated by the concomitant effects of general anesthesia. The data on the effects of the various anesthetics per se are lacking in many details. A possible synergic action of the drugs, which is entirely different from the action of either, may exist. Just what this action may be cannot safely be predicted. That anesthesia does affect the adrenin reaction has recently been shown in this laboratory by Berry (1), in his study of the effects of adrenin on the blood pressure reaction of the unanesthetized dog. The most striking effect observed was that ether anesthesia markedly depressed the blood pressure reaction to adrenin. The question then arises, Is this depression exerted on the vasomotor system as a whole or as is entirely possible, are the vasoconstrictor nerves selectively affected?

The extent of the data on the adrenin problem has reached such proportions that it would seem that the time is at hand for more intelligent generalization on the rôle played by the drug in the various normal and pathological activities of the body. Before such generalization can safely be attempted, however, the possible variable of anesthesia must be eliminated. The whole problem of the pharmacology of adrenin in the unanesthetized animal is therefore in need of study. Certain aspects of this problem are now under investigation in our laboratory. The first specific function to be considered is that of the effects of adrenin on the renal secretion.

The generally accepted action of adrenin on the secretion of urine is that of marked inhibition. The recorded literature on the subject, however, does not conclusively support this fact. Bardier and Fraenkel (2), who were the first to study the effects of adrenal extracts on the

urinary secretion, observed a marked reduction in the amount of urine passing from the ureters during an injection. With larger doses there was complete cessation of urine flow. These reactions were then followed by marked polyuria which lasted for some considerable time. Occasionally the injections were followed at once by polyuria. Their experiments were made on anesthetized dogs apparently under the influence of curare. Their extracts were made either from desiccated glands or from fresh glands macerated for twenty-four hours at body temperature. Judging from the effects on the arterial pressure, relatively large doses of the drug were employed. Whether the use of curare or the presence of protein decomposition products in their extracts played any part in their results was not determined. Schlayer (3) in his study of experimental nephritis observed that adrenin, when administered intravenously to animals containing much fluid, acts under certain conditions as a diuretic. In some unpublished studies on the effect of adrenin on the intestinal and renal secretion of the dog during hydraemic plethora, we failed to observe this effect. After repeated injections of varying dosages of adrenin the urinary output was decreased and was often one-fifth to one-tenth of that of the small intestine. Schlayer used large doses of adrenin without blood pressure control and the majority of his animals were renally abnormal. Biberfeld (4) reported that the subcutaneous injection of adrenin in doses of 1.5 to 2.5 mgm. per kilo, produces a marked diuresis in rabbits. Pollak (5) also observed diuresis to occur from adrenin injection. Sollman (6) observed that in the perfused kidney, when adrenin was added to the perfusate to make a dilution of 1-50,000, there was a marked decrease in the rate of urine flow. The concentration of adrenin used by this investigator, however, was such as to more nearly produce a toxicologic rather than the physiologic action of the drug. Meltzer and Auer (7) observed that adrenin restricts elimination and suggested that the substance probably interferes with the eliminating power of the kidney. Again, however, the effects of fairly large doses of the drug were studied whereas there is every probability that under physiologic conditions only minute quantities are ever present in the blood stream. Kleiner and Meltzer (8) later, in an extended comparison of the effects of subcutaneous and intramuscular injection of adrenin in rabbits, showed that the absorption of the drug must be at a very slow rate to produce diuresis. Diuresis occurred only with subcutaneous doses of 0.7 to 1.0 mgm. per kilo. Intramuscular doses of this size or larger doses subcutaneously were found to inhibit the flow of urine. Cow (9)

established the anatomical relationship of an anastomotic branch of the adrenal vein with the cortex of the kidney. From a series of perfusion studies he concludes that under certain conditions adrenin in appreciable amounts is poured directly into the kidneys from the suprarenal bodies of the intact animal, producing a diminution in the flow of urine.

Failing to find reported in the literature the effects of threshold dosages and of slow infusions of adrenin on the urine flow, which probably more nearly approach the normal discharge of the gland, it was necessary to study the effects of these dosages in the anesthetized dog for comparison with their effects in the unanesthetized animal.

METHOD

Medium sized dogs were used as experimental animals. Quiet, good-natured dogs were selected and great care used to avoid exciting them. After some petting, the dog was carefully and quietly laid on the operating board and strapped back down. In the early experiments one-eighth to one-fourth of a grain of morphine was administered but later with careful handling this was found to be unnecessary. One to two cubic centimeters of 2 per cent cocaine solution or an equal amount of 1 per cent quinine-urea bimuriate solution were injected intradermally over the femoral artery and vein just below Poupart's ligament. After waiting a few minutes for the anesthetic to take effect, arterial and venous cannulas were set. Next the skin and subcutaneous tissue over the lower median line of the abdomen were injected with the anesthetic. Again after waiting a few minutes an incision 5 or 6 cm. in length was made into the abdominal cavity, beginning at the upper border of the pubic bone and extending cephalad. The urinary bladder was then aspirated if necessary and drawn through this incision. The ureters were each isolated and cannulated close to the bladder and the viscus was replaced in the abdomen. The abdominal incision was then closed, leaving the cannulas protruding from the wound. All manipulations were made with as little trauma as possible.

The ureteral cannulas were led into a Y tube which extended over the edge of the table and the system was filled with water or salt solution. The blood pressure was recorded from the femoral artery with an undamped mercury manometer. The ureteral outflow was recorded in drops by means of a key and signal marker. Adrenin (Parke, Davis and Company's "Adrenalin") in varying doses was injected into the femoral vein, using a small amount of warmed 0.8 per cent sodium chloride solution to flush it in.

RESULTS

Rapid absorption of the cocaine solution was found often to make the animals very irritable. In one case the dog died in convulsions on the table. The observations made under cocaine anesthesia were therefore discarded. Quinine-urea bimuriate in 1 per cent solution was substituted. The perfect anesthesia resulting from its use was remarkable. The duration of the experiments was from three to six hours. Quite often the dogs would sleep throughout the greater part of this time.

For comparison, after a number of reactions had been recorded on the unanesthetized dog, ether was administered and the injections repeated. The effect of ether on the urine flow was very striking. As soon as the animal commenced to struggle against the anesthetic, the urine flow immediately ceased. The return was very slow requiring some fifteen to thirty minutes. In view of the following observed effects of adrenin on the urine flow, this might be interpreted as due to a discharge from the adrenal glands. It was not due to the struggling alone for in one dog that was sleeping the administration of ether occasioned a marked diminution in the urine flow without any struggling. The inhibitory influence of ether on the urine flow may well be due to adrenin for Elliott (10) has shown that ether produces discharge of the adrenal glands. On the other hand it must be recognized that this is only one of the various possible explanations. The recent work of Stewart and his collaborators (11) must make us cautious in ascribing to the adrenal glands the causation of reactions which can be explained on other grounds.

The effect on the urine flow of adrenin in all effective doses in both anesthetized and unanesthetized dogs was that of marked inhibition. In the unanesthetized animal the threshold for the reaction was found to be lower than after ether anesthetization. Figure 1 shows the results of an injection of 0.5 cc. of a 1-200,000 dilution of adrenin with practically a negative blood pressure reaction but with more than 50 per cent reduction in the urine output. This is the characteristic effect of injections of all dosages. The injection of larger doses produces a complete cessation of urine flow until ten or twenty seconds after the blood pressure has returned to normal. Figure 2 shows the effect of a pressor infusion. During the greater part of the blood pressure reaction there is practically a complete cessation of the urine flow. The normal outflow returns at about the same time the blood pressure

reaches normal. This type of reaction was observed with all strengths and rates of infusion, the degree of urinary inhibition varying directly with the concentration and rate of infusion.

The effects of infusions lasting fifteen to thirty minutes gave results which were somewhat striking. In dog no. 14 an infusion lasting thirty minutes and thirty seconds was started while the urine flow was approximately one drop in five seconds. During the first sixty-five seconds of the infusion, four drops of urine flowed. From then until the infusion was shut off, (twenty-nine minutes and twenty-five seconds,) but four drops flowed. Eight minutes after the infusion was shut off

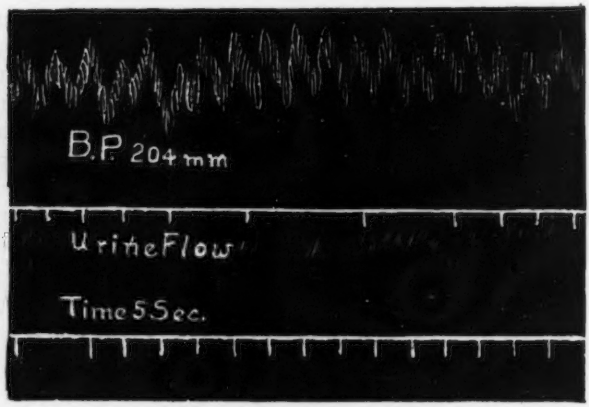

Fig. 1. Graph showing effects of threshold adrenin injection on urine flow and femoral blood pressure of an unanesthetized dog. Dose, 0.5 cc. of 1–200,000 dilution. Time, five seconds. No reduction. Dog weight, 14 kilos.

the urine returned to and remained normal. The quantity of the infusion was 30 cc. of a 1-50,000 solution of adrenin. There was a blood pressure rise of 20 mm. of mercury (from 120 mm. to 140 mm.). The most striking feature of such observations was that the urine flow should so soon return to normal after practically an anuria of so long a duration. It would seem to suggest that adrenin produces the marked inhibition on the urine flow in a way other than by the ischemia of renoconstriction. It is possible as Meltzer and Auer (7) have suggested that the substance in some way interferes with the eliminating power of the kidney and the action may be on the secreting cells directly. If this be true, the action is merely inhibitory for repeated injections

of the drug during an experiment did not permanently decrease the flow of urine.

Considerable attention was directed toward threshold dosages of the drug to detect the appearance of any diuretic effect but none was observed.

It was observed that when 10 to 20 cc. of the "flush in" solution were used with an adrenin injection, an "over-recovery" occurred in the urine flow. When smaller amounts of the "flush in" solution were used and with infusions when no "flush in" solution was used, this "over-recovery" was not observed and was due, therefore, either to the slight degree of hydraemic plethora induced by the salt solution or to a direct stimulation of the renal cells by the sodium chloride.

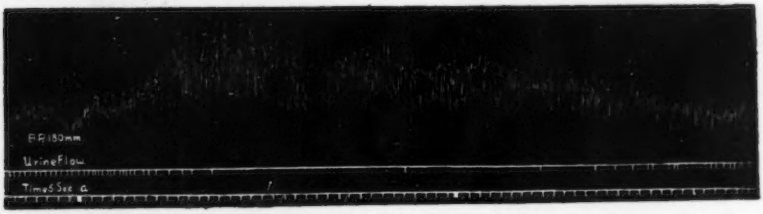

Fig. 2. Graph showing effects of adrenin infusion on urine flow and femoral blood pressure of unanesthetized dog. Dose, 20 cc. of adrenin 1-200,000 in one hundred and ninety seconds, from *a* to *b*. Time, five seconds. Reduced to one-third. Dog weight, 7 kilos.

SUMMARY AND CONCLUSIONS

1. Adrenin in all effective dosages administered intravenously inhibits the urine flow in both anesthetized and unanesthetized dogs.

2. The threshold of the reaction is slightly lower in unanesthetized than in anesthetized animals.

3. Small injections and infusions merely inhibit the flow of urine while larger doses produce a complete cessation of flow.

4. The inhibition usually persists until shortly after the blood pressure reaction is complete.

5. Diuresis succeeding the inhibition was not observed.

6. The rapid return of the flow to normal after prolonged infusions suggests that the drug exercises its inhibition on the kidney function in a way other than by the ischemia produced.

7. During the administration of ether the urine flow is completely checked and recovery under the anesthetic takes place slowly.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

Thirtieth Annual Meeting

Minneapolis, December 27 28, 1917; Rochester, December 29 1917

Some phases of industrial fatigue. Frederic S. Lee (for the Committee on Industrial Fatigue).[1]

The investigation of industrial fatigue was assigned to the author by the Committee on Physiology of the National Research Council. The Committee on Industrial Fatigue was thereupon organized under the Advisory Commission of the Council of National Defense and became associated with the U. S. Public Health Service, which appointed to positions on its staff several members of the Committee and appropriated a sum of money to defray the expenses of the work. Since July, 1917, the Committee has been engaged in an investigation of munit on factories from the physiological standpoint, with two aims in view: the more purely scientific one of learning how the human machine works in industry; and the practical aim of discovering the condi ions under which it can work most efficiently, and endeavoring to secure the establishment of these conditions in factories that are manufacturing war supplies. While visiting and observing the work in many places, two leading factories were selected for intensive study; a brass factory which was engaged largely in making fuses for explosive shells; and an automobile factory which was devoting a considerable part of its resources to government work. In the brass factory, the day shift works ten hours in two spells of equal length and has one hour's interval for luncheon; the night shift works twelve hours and has twenty minutes for luncheon immediately after midnight. In the automobile factory there is an eight hours' working period divided into two spells by a luncheon period of about one half hour. Especial attention has been given to studying quantitatively the output of the individual during each hour of the working period. More than thirty different operations, involving repetitive work, have been studied and from thirty to several hundred observations in each have been made.

The curve of output has been found to vary with the character of the work. Where close attention and exact muscular coördination are required, the average output curve of the individual during the first spell

[1] The Committee on Industrial Fatigue: Thomas Darlington, Chairman; Frederic S. Lee, Executive Secretary; Robert E. Chaddock, Raymond Dodge, David L. Edsall, P. Sargant Florence, Miss Josephine Goldmark, Ernest G. Martin, Ernest L. Scott and J. W. Schereschewsky.

resembles that of a single excised muscle stimulated for a given period by a series of single induction shocks. In both there occur in succession a rise (treppe in the muscle, "practice effect" in the human being), a maximum, and a fall indicating fatigue. In operations that are markedly muscular, though here further observations are desired, there appears to be a steady fatigue fall from the first, with little or no practice effect, but often with a minor rise and fall ("spurt") just before the end of the spell. In monotonous operations involving frequent pauses, the curve after a slight practice effect becomes horizontal, with no fatigue fall. During the second spell, after rest and food, the curves are repeated as to their general form, but there appears at first a recovery of working power, if fatigue has resulted from the work of the first spell, and the final fatigue fall is more pronounced that at first. The operations that have been studied during the night shift of twelve hours reveal curves in general like those of attentive work, but during the last two hours output falls off greatly and during the final forty minutes often nothing whatever is produced.

Wherever the output curves show a pronounced fall as the spell proceeds, the introduction of rest periods is indicated, and the Committee has therefore recommended that in such cases each spell be broken by a ten minutes' resting interval. This has been done with certain groups of workers in the eight hour shift with the result, in especially fatiguing operations, of increasing the output by 6 to 8 per cent. The Committee further recommends that the twelve hour night shift be shortened by two hours, and this is about to be established experimentally with, it is hoped, no diminution and possibly an actual increase, of the total night's production.

The Committee finds very prevalent an output that is almost or quite uniform from day to day and week to week, even where piece-rate wages prevail. It is often far below individual capacity and perhaps represents a physiological defense of the individual against overdriving. How this "stereotyped" output may be eliminated and production be raised more nearly to physiological capacity is receiving the attention of the Committee.

The Martin spring balance muscle test, now used for the first time in industrial work, has shown that it is capable of revealing fatigue in the individual and also that each factory operation has its own standard of strength. The Committee recommends its use in factories in classifying workers and assigning them to their appropriate tasks and as a general test of the physical condition of workers from time to time.

The Committee has also made observations on certain chemical changes occurring in fatigue, on the rhythm of individual workers, on the course of accidents throughout the working spell, on the problem of labor turnover, and the occurrence of minor illnesses in workers, but their conclusions on these matters are not yet ready for publication. The work is still proceeding.

On Weichardt's so-called "fatigue toxin." FREDERIC S. LEE and B. ARONOVITCH.

When juice is pressed out of the muscles of fatigued animals and injected into other animals there result stupor and a temporary lowering of the body temperature. Such fatigue juice when administered to excised muscles causes a marked decrease in their working-power, as shown by a shortening of the working period and a decrease of the total work performed.

Juice from the muscles of non-fatigued animals exerts exactly the same action as fatigue juice, on both animals and excised muscles.

The experiments thus fail to confirm Weichardt's assumption of the existence of a specific toxin of fatigue. This conclusion is supported by an examination of Weichardt's writings, which shows that he uses the terms "toxin" and "antitoxin" vaguely and not in accord with the present use of serologists.

The quantitative measurement of general fatigue. A. H. RYAN (with the collaboration of J. H. Gordon).

The present experiments were begun in a search for further methods of measuring fatigue. The following tests were employed; visual acuity, as recently used by Stanley Kent; copying test, in which simple text was copied for a period of ten minutes, the characters written, and number of errors being computed; systolic and diastolic blood pressure; and the vascular skin reaction as described by Marey, consisting of the appearance of a white line on the skin, after the skin is gently stroked with a blunt instrument. The stroke was made on the flexor surface of the forearm, and sometimes on the chest and back, and the white streak which occurred was studied, in regard to latent period, time required for reaching the maximum intensity, time at which it began to spread and fade, and the time of complete disappearance. An instrument was devised by which the stroke could always be made with the same pressure, adapting the principle of Grützner's "myographion."

In the first series of experiments on a group of five hospital nurses, lasting three weeks, the observations were made in the morning, at noon, and in the evening, after meals. In regard to the visual acuity, the copying test, and the blood pressure, no definite relation could be established between work, rest in the afternoon, and loss of sleep, and the results of the tests. In regard to the vascular skin reaction it was found that the length of time between the making of the stroke, and the beginning of fading of the streak, fading time, was always decreased by work and activity, and increased by rest or sleep. The recovery at night was less if the night's rest was poor.

In several subjects climbing a hill for half an hour, there always resulted a definite decrease in the fading time of the reaction, which was recovered from, by reclining. Partial recovery from strenuous work was observed during the progress of lighter work.

Daily observations on the vascular skin reaction in one subject, for two weeks, at one and a half hour intervals during the day, showed a

continuous decrease in the fading time of the reaction during the day, in proportion to the severity of the work. Partial recovery occurred during lunch hour, and whenever the subject rested.

A short series of control experiments were made upon the effect of temperature; also upon subjects resting throughout the day. The influence of barometric pressure was ruled out.

Four of the nurses had "colds," and one had a recurrence of fever, following an old tuberculous peritonitis. These conditions were either preceded by, or occurred simultaneously with, a low level in the fading time of the reaction.

The following observations were made relative to the mechanisms concerned in the production of the streak. First, when a certain subject was "chilly," "goose-flesh" could be obtained wherever the stroke was made, and its appearance considerably preceded the appearance of the white streak, and disappeared sooner. Second, a white streak which has almost completely faded, may be revived by making a stroke in a neighboring skin area. Third, embarrassment may result in the appearance of a red streak preceding the white streak. This was first observed in a girl who had made a misstatement regarding a question in her history of the previous day.

Strength tests in industry. E. G. MARTIN.

Tests of muscular strength, according to a method previously reported, were made on operatives in munition factories. The strength is determined by measuring with a dynamometer the maximum resistance to a pull which overcomes the contraction of the muscle-group under examination. To make the test practicable for industrial conditions it was abbreviated by using only five pairs of muscle-groups (pectorals, flexors of forearm, flexors of wrist, adductors of thigh, abductors of thigh), and computing from these the entire bodily strength. Previous investigation had shown that this computation is accurate within an average range of 5 per cent.

The following results were obtained:

The normal non-athletic individual tends to show a strength whic is roughly twenty-five times his weight. The chief exceptions will be found among very light and very heavy individuals.

In industrial operations calling for the exercise of strength there is a pronounced tendency toward a standard strength for each job. In other words, operations in which a definite exertion is required tend to develop a degree of strength determined rather by the exertion than by the weight of the worker.

Comparisons of strength at the beginning and end of the work-shi t showed that in general there is in the easy jobs a tendency to make a better showing at the end than at the beginning. In the operations requiring moderate exertion the stronger workers do better at the end than at the beginning, but the weaker workers are likely not to do so well at the end as at the beginning. In the hard jobs all the workers tend to do less well at the end than at the beginning.

Comparisons of the daily strength records of workers with their daily production records show that on days in which good strength records are made production is likely also to be good, and on days in which poor strength records are made output is likely to be poor.

Nutritive factors in some animal tissues. LAFAYETTE B. MENDEL and THOMAS B. OSBORNE.

Rats fed on a diet in which skeletal bee muscle formed the sole source of protein and water-soluble vitamine grew for a few days and then declined. Addition of small amounts of yeasts to the food promoted rapid recovery and growth. Accordingly such muscle contains protein suitable for nutrition but is deficient in the water-soluble vitamine.

Beef thoroughly extracted with water and fed as the sole source of protein and water-soluble vitamine in an otherwise adequate food mixture failed to promote growth; animals thus fed declined immediately. The addition of small quantities of yeast, corn germs, wheat embryo, "protein-free milk" or dried liver to this diet checked the decline and induced rapid growth, showing that the protein of the extracted meat was adequate for growth, but that the water-soluble vitamine was lacking.

A sufficient quantity of the water extract of meat may furnish enough water-soluble vitamine to promote considerable growth.

Dried liver and dried heart suffice as the sole source of protein and water-soluble vitamine in an otherwise adequate dietary. Whether they can furnish sufficient fat-soluble vitamine is being determined.

Further observations on the production of lactic acid following alkaline injections. J. J. R. MACLEOD.

The immediate increase in the lactic acid content of blood produced by injections of sodium carbonate sufficient to lower C_H raised the question as to whether the function of acid production might be to neutralize the alkali. Production of lactic acid out of carbohydrate would then serve as an acid reserve of the acid base equilibrium of the body. If such should be the case, much of the acid would be excreted with the urine, and both in this secretion and in the blood no perceptible change in C_H of the blood would necessarily occur.

The following experments bearing on this aspect of the problem have been partially completed.

1. When 5 per cent sodium carbonate solution was injected intravenously in etherized rabbits at the rate of 1 cc. per minute, it gradually raised P_H of blood from 7.4 to 7.8 in about three hours, without having any marked effect on the respiratory rate. The urine was freely excreted, and the percentage of lactic acid rose from the normal of about 0.01 to over 0.30. A total of 7.5 grams Na_2CO_3 was injected, and a total of 0.654 gram lactic acid excreted. Between 5 and 6 had therefore been excreted as sodium lactate.

2. In a similar observation on dogs, the percentage of lactic acid in the urine rose in one case from 0.028 to 0.201, and in another from 0.045

to 0.495. In the former case 2 per cent Na_2CO_3 was injected (1 cc. per minute), and P_H of blood rose to 7.9 in four hours. In the latter case 1 per cent Na_2CO_3 was injected, the P_H rising to 7.6 in three hours. Diuresis was present in both cases.

3. An etherized dog was injected with 1 per cent Na_2CO_3 at the rate of 2.5 per minute, receiving in all 4 grams Na_2CO_3 in two and three-fourth hours. P_H of blood remained at 7.4. The CO_2 percentage rose only slightly and a total of 0.392 gram lactic acid was excreted by the urine. Between 5 and 6 per cent of the administered alkali was therefore excreted as lactate.

4. The daily urine was collected in a cat that was fed by stomach tube with a regular daily ration of minced liver and flour. After a preliminary period of eight days, sodium carbonate was added to the food for a further period of five days, this being followed by another normal period. During the alkali period, P_H of the urine rose from 6.1–6.3 to 8.2–8.3. The volume rose somewhat, and the relative percentage of ammonia-nitrogen fell from about 4 to less than 2. The total daily excretion of lactic acid during the normal periods averaged 5.5 mgm. (varying between 3.55 mgm. and 7.3 mgm.); during the alkali period the average was 10.5 mgm., being much greater (0.022 mgm.) on the first few days than subsequently. A similar result was obtained on a cat given bicarbonate in place of carbonate.

5. No difference was observed in the percentage of lactic acid in the daily urine of three rabbits according to whether they were starved for several days or fed with cabbage. The total excretion for twenty-four hours was greater in the case of the fed animals, but the very much greater water excretion of these as compared with the starved animals probably explains the difference.

6. No difference was observed in the percentage or in the total amount of lactic acid in the daily urine of rabbits fed liberally with cabbage with or without alkali.

7. Administration of sodium bicarbonate in therapeutic doses in man caused a distinct increase in the excretion of lactic acid which was most marked in the urine of the day following the administration.

(*a*) *The isolation and identification of the thyroid hormone;* (*b*) *The physiological action of the thyroid.* E. C. KENDALL.

The method for the isolation of the iodin-containing compound of the thyroid was briefly reviewed, and its empirical formula shown to be $C_{11}H_{10}O_3NI_3$, and its structural formula has been established as trihydro tri-iodo oxy-indol propionic acid. The oxy-indol group can change to the hydroxyl, and does change when the compound is dissolved in an alkaline solution.

Physiological testing has shown that this substance will relieve all symptoms of cretinism and myxedema to the same extent as desiccated thyroid. Furthermore, there is no relief of the conditions of cretinism and myxedema by all of the constituents of the thyroid other than this substance.

From a consideration of its structural formula, it is possible to relate its physiological activity to chemical reactions produced by the imino group of the indol nucleus and the carbonyl group. It has been shown that this substance will react in vitro with amino acids; that the carbonyl group condenses with the amino group of amino acids, and that the imino group is exceedingly reactive with the carboxy. It seems probable that the physiological activity of the substance is due to the action between itself and the amino acid. There may be three results of this action, (1) deaminization, (2) decarboxylation, and (3) both. To show the reaction of these two groups, the imino and carbonyl, derivatives of each were made. It was found that the animal organism could utilize the substance when the carbonyl group had reacted forming a nitrogen derivative, and this reaction presumably is involved in the deaminizing action of the substance in the body. However, replacing the hydrogen of the imino group with derivatives renders the substance inert within the animal organism. Hence, it is improbable that this action occurs in the functioning of the compound within the body. This reaction shows also that when the imino group can not function, the entire compound is rendered physiologically inert. Bearing in mind this physiological activity, the compound has been named "thyro-oxy-indol." This word, being too long to extend to every-day use, has been abbreviated to "thyroxin."

Evidence was submitted which supports the hypothesis that this substance is the only compound in the thyroid secretion which is essential for the production of thyroid activity in the body, and hence it may be considered the thyroid hormone.

The influence of music upon electrocardiograms and blood pressure. I. H. HYDE and W. SCALAPINO.

The object of the experiments of which only the beginning is here briefly summarized, is to ascertain the effect of different kinds of music upon the heart and blood pressure, in individuals who are known to have musical talent, and are fond of music, also in persons who are indifferent and have no fondness for music, in neurasthenics and in some animals.

The effects of the three following pieces of music were investigated, Tschaikowsky's death symphony, characterized by its tragic slow minor movements; Toreador's brilliant description of the bull fight, from Carmen, and the National Emblem, a stirring rhythmical march by Sousa. The experiments were conducted on three men who are fond of music. The data from subject "A" being more extensive and quite typical are here presented.

The cardiograms were obtained with an Einthoven string galvanometer, and the blood pressure with a Tycos and modified Erlanger sphygmomanometer. The records were taken before, during and after listening to the music.

A study of the cardiograms and tabulated results, revealed first, that the effects of the minor tones of symphony music, were a fall in

systolic, diastolic, and pulse pressure, and increase in the pulse rate, and also an increase in the E. M. F., or action current. The results were probably due to a reflex inhibitory action on the vagus nerve.

Second. Toreador's stirring song produced an increase in the systolic and pulse pressure, but a decrease in the diastolic pressure. The pulse was accelerated and the E. M. F. of the ventricular contraction was lessened. It may be, that this kind of music reflexly stimulates the accelerator or inhibits the vagus.

Third. The effect of Sousa's rhythmical march was increased systolic and pulse pressure, a decrease in the pulse rate but an increase in the action current. The effect seems to be due to vagus stimulation, and it is possible that this as well as other kinds of music may have a physiological influence on the system in other respects, and that by a careful selection of music from a definite source, prove an aid in the treatment of nervous disturbances.

A simple method for the resuscitation of the human heart. ARTHUR D. HIRSCHFELDER.

Observation of exposed dogs' and cats' hearts shows that death ensues in these ways—either the ventricles go into fibrillation, or they cease to contract and at once lose their irritability entirely, or they may cease to contract and yet for some time retain their ability to carry out a forcible contraction in response to mechanical stimulation. Such a stimulation may be furnished by directly slapping the ventricles with a blunt instrument or by suddenly pressing the ribs and chest wall down upon the heart. If this is done rhythmically at a rate of thirty to sixty times a minute the heart may respond to each stimulus, the circulation may be reëstablished, and spontaneous cardiac rhythm may then be resumed. The writer carried out this procedure successfully in one case of stoppage of the ventricles in a patient with Adam-Stokes' syndrome in which the stoppage of the ventricles was unusually prolonged. The convulsion and respiration had entirely ceased and the patient seemed beyond hope of recovery. The ribs and chest wall were then seized between the two hands, the right hand at the back, the left hand over the precordium, and the chest was forcibly compressed between them at a rate of thirty to forty times a minute, thus pressing ribs and chest wall suddenly down upon the ventricle. The heart responded at once to each stimulus with a contraction, circulation was rapidly resumed, the patient recovered and was living two years afterward. This manoeuver has been repeated as a routine upon the hearts of dogs and cats used in the laboratory experiments in which the hearts had been exposed by removal of part of the chest wall, and where the results could be watched with the eye. It is often successful in restoring the circulation. The rather slight stimulus offered when the chest wall is pushed suddenly down against the heart, without in the least subjecting the latter to compression, suffices as a mechanical stimulus to bring about contraction. This manoeuver is, therefore, a justifiable

procedure for the resuscitation of hearts which have stopped beating for a minute or more, and which are apparently beyond hope of the ordinary forms of stimulation. It is simpler and more readily applied than massage across the diaphragm or electric stimulation after the introduction of a needle into the heart.

Regulation of venous blood pressure. D. R. HOOKER.

The experiments were performed on dogs and deal especially with the reflex control of venous tone. The sigmoidal region of the large intestine was isolated as to its blood supply, double mass ligatures above and below the area interrupting collateral circulation and permitting elevation of the preparation above disturbing influences of neighboring viscera. The vascular bed was washed free of blood with warm Ringer's solution after which the artery was left freely open. It was assumed that arterial constriction would displace fluid backward rather than forward into the vein where the pressure was 10 to 15 cm. H_2O. The mesenteric vein was connected with a water manometer. A similar manometer was connected with the lumen of the isolated gut which had been previously filled with warm Ringer.

Stimulation of the nerve trunk running from the inferior mesenteric ganglion gave a rise of pressure in the vein amounting to 7.25 cm. H_2O in a number of cases. This reaction was readily and repeatedly obtained for periods of an hour or more. Stimulation of the saphenous nerve and asphyxiation gave rises of 3.5 cm. and 4.5 cm. respectively. The latter results were much less easily obtained, failing long before the peripheral mechanism was exhausted. Section of the peripheral nerve or destruction of the medulla destroyed the reflex. These venous pressure changes were independent of pressure changes in the lumen of the gut.

The evidence points to the existence of a central as well as a peripheral veno-pressor mechanism. The possible relationship of the mechanism to "shock" has not as yet been studied but the fact that the central mechanism loses function so easily suggests that it may be one of the early events contributing to the stasis of blood which is regarded as a cardinal symptom in the "shock" complex.

Blood pressure in sharks and the shock problem. E. P. LYON.

Blood pressure in sharks was studied on account of the peculiar circulatory arrangements. The blood pressure is about 35 to 40 mm. in the branchial arteries and 20 to 25 mm. in the systemic arteries. Under experimental conditions there is a slow fall of pressure through a long time, with corresponding gradual loss of reflexes and other activities. The equilibrium functions seem to be the first to disappear and the heart reflexes last, respiration being usually lost before the heart reflexes.

Many mechanisms which are effective in producing shock in dogs have little or no effect on sharks. These fish are remarkable in that stimulation of almost any part of the body causes inhibition of the

heart. There is an intimate relation between the respiratory and heart rates, a 1-1 rhythm being common, while at other times a 2-1, 3-1 or indefinite relation subsists. Artificial respiration (by a stream of water led into the mouth), produces variations of heart and respiratory movements. In a considerable number of animals change from light to dark lowers the blood pressure.

Observations in shock. C. C. GUTHRIE.

Dogs under ether anaesthesia were reduced to a state of shock chiefly by nerve stimulation alone, and combined with partial cerebral anaemia. Typically, the condition was associated with low blood pressure, decrease of respiratory movements, presence of eye reflex and absence of pronounced tendency to recover on discontinuing anaesthetic. Marked differences in resistance occurred in different animals as well as marked differences in the quantitative relations of the symptoms and in reflex functional response in shock. Also, marked quantitative variations occurred in chemical studies, such as the amount of oxygen retained and the amount of CO_2 eliminated by the lungs, the hydrogen ion concentration of the blood, and blood plasma bicarbonate.

Among the direct negative findings, as primarily causative of shock, were blood pressure; blood reaction, including reserve alkalinity concentration of chemical constituents or morphological elements, and viscosity of blood; blood volume; pooling of blood in vessels of alimentary canal and liver; temperature; cardiac weakness and cerebral embolism.

The amount of oxygen retained and CO_2 eliminated by the lungs was less in pronounced shock, but there is no reason for assigning to these conditions a causal relation to shock.

That acidosis was not a primary causative factor was shown by failure to produce shock by primary acidosis, as by injections of lactic acid; recovery from fatal lactic acid intoxication by injection of sodium bicarbonate, and absence of recovery from shock by such injection; and production of shock with alkaline reserve maintained by sodium bicarbonate injection.

Among phenomena observed possibly having causal relations to shock, were alterations in nervous activities, particularly fatigue of bulbar centers. Though presenting marked variations, results obtained in pronounced shock showed that both reflex vasomotor and respiratory response may be profoundly decreased. Interpretation of some of the reflex vasomotor phenomena is difficult.

Evidences point to decreased arterial and increased venous blood volume in shock, and derangement of the veno-motor mechanism may have an important causal relation to the condition.

Shock and its control. W. B. CANNON.

The observations were made at a casualty clearing station in France a few miles back from the front-line trenches. The main points may be summarized as follows:

1. There is a discrepancy between the red counts, haemoglobin and haematocrit readings of venous and capillary blood in shock cases, indicating a concentration of blood in capillaries. It seems probable that the "lost blood" of shock is in capillary areas.

2. The shock cases when received at the clearing station, have a diminished alkali reserve (an acidosis, in the Van Slyke sense).

3. A rough correspondence exists between the degree of acidosis and the degree of depression of the blood pressure.

4. Surgical operation in any case lowers the alkali reserve, and in these shock cases, with an acidosis already existent, operation may in a short time reduce the reserve to a serious degree.

5. In shock cases, surgical operation not only causes a sharp fall in an already low alkali reserve, but also a sharp fall in an already low arterial pressure. Thus two conditions unfavorable to recovery are made worse by the necessary operation.

6. By injection of sodium bicarbonate intravenously *at the start of operation* both of the unfavorable effects of operation are obviated—the pressure is higher at the end than at the start, and the alkali deficiency is abolished.

Observations on the volume flow of blood through the submaxillary gland. ROBERT GESELL.

The volume flow of blood through the submaxillary gland was studied under normal conditions, and under conditions of lowered blood pressure induced—(1) by hemorrhage, and (2) by tissue abuse.

Under normal conditions with a constant blood pressure, the basal flow of blood remained constant during periods of glandular rest.

On increased glandular activity, elicited by chorda stimulation, there was increased volume flow of blood which bore a linear relation to this increased activity.

Lowering of the blood pressure by hemorrhage reduced the ratio of secretory blood flow to secretion, and also reduced the basal volume flow of blood.

The initial fall in basal flow of blood was very much more rapid than the accompanying fall in blood pressure. This initial fall gave way to a fall in volume flow much slower than the accompanying fall in blood pressure. At a pressure of about 50 to 40 mm. Hg., the fall in basal flow of blood again became decidedly faster than the fall in blood pressure.

Lowering of the blood pressure by tissue abuse likewise lowered the ratio of secretory blood flow to secretion. In one experiment the secretion of one drop of saliva, at a blood pressure of 124 mm. Hg., called forth 13.9 extra drops of volume flow of blood through the gland. At a pressure of 37 mm. Hg. no extra flow of blood occurred. The basal volume flow of blood likewise fell; but more rapidly with the initial decrease in blood pressure than in the case of lowered pressure from hemorrhage. In one instance, the basal flow of blood at a pressure of 84 mm. Hg. was only 16 per cent of that obtaining at a pressure of 124 mm. Hg.

The curves of basal flow of blood were explained on the basis of the variations of three factors—viscosity of the blood, driving pressure, and caliber of the vessels.

The differences in the curves of basal flow obtained in the two types of experiments can probably be largely explained by the direction of change in viscosity of the blood.

In the case of hemorrhage, there is a decreased viscosity counteracting the effects of the accompanying fall in blood pressure; while with tissue abuse, there is an increased viscosity[1] augmenting the effects of decreasing blood pressure.

The experiments show the unfavorable conditions produced by lowered blood pressure for the maintenance of normal conditions, especially if any tissue is called upon for increased activity.

They show the gravity of even a small fall in blood pressure, for it is the initial fall in pressure which produces the greatest decrease in basal volume flow of blood.

Some reactions in the development of shock by diverse methods. JOSEPH ERLANGER, ROBERT GESELL, H. S. GASSER and B. L. ELLIOTT.

Shock supervenes in consequence of extensive tissue damage, not necessarily traumatic in origin. It appears after partial occlusion of the inferior vena cava, and of the descending aorta; after plugging the portal capillaries with lycopodium; and after large doses of 1-1000 adrenalin; in other words, after interfering for some time with the blood supply to a part, or the whole, of the body. At autopsy there are haemorrhages into many of the abdominal organs. The picture is similar when shock is produced by exposure of the intestines; and in addition a considerable quantity of plasma transudes from the serous surface. That there is a similar plasma transudation, but into the tissues, during the development of the other forms of shock, is indicated by the fact that in all there occurs a diminution in plasma volume; and in addition there is often a reduction in the volume of the blood as a whole.

The reaction of the vasoconstrictor center, followed by a modification of Bartlett's inflow method, depends largely upon the effect of the procedure by which shock is produced upon the cerebral circulation. The tone of the center at first is inversely as the cerebral arterial pressure, but long continued low pressure always leads eventually to loss in tone. It is possible to have shock with normal vasoconstriction.

The force of the heart beat seems to diminish somewhat as shock develops, but presumably only secondarily. Furthermore, late in shock, lowering the arterial pressure may paralyze the respiratory center without preliminary stimulation. The alkaline reserve declines as shock develops. The decline though may be extreme or not below the normal range.

[1] Gasser, Meek and Erlanger: Proceedings in this Journal.

The mechanism of shock, we conclude, is as follows: Extensive traumatization causes extensive local transudation of plasma, which, together with the primary haemorrhage, materially reduces the blood volume, and thus leads to general vasoconstriction which is enhanced reflexly by the pain. The blood stream is thus slowed to the point of damaging the cells, and of starting generalized transudation. The arterial pressure as a result eventually becomes so low, that the vasoconstrictor center suffers and the arterial pressure falls still further. At the same time, the alkaline reserve diminishes, possibly through the incomplete oxidation of metabolites. Thus a series of vicious cycles is started, the outcome of which is "Shock." Sometimes one, sometimes another of these three factors predominate.

At the front, fatigue entailed by transportation of the wounded, contributes toward the ultimate giving way of the vasoconstrictor center; it presumably works in the same way as does ether in laboratory experiments. If there is such a thing as traumatic shock in the absence of wound weeping and blood loss, we would suggest in explanation of it, that long continued pain stimulates the vasoconstrictor center, and so diminishes the blood supply to the tissues to the point where transudation begins, thus starting the vicious cycle just described. Naturally any other set of conditions tending to hold the arterial pressure low for some time, also would establish the same vicious cycles.

A method for the determination of blood volume. WALTER J. MEEK and HERBERT S. GASSER.

A method for the determination of blood volume has been devised which is free from certain objections incident to the older methods. The general plan has been to inject into the blood stream some substance which was inert, which disappeared slowly, and which could be recovered quantitatively. A determination of the dilution of this substance in the blood would then afford data for the blood volume determination.

Acacia has been found to meet the above requirements. A given amount is added to blood in vitro for a standard or control. A given amount is then injected into the animal. Ten minutes later a sample of blood is drawn for determination. The amount of dilution indicates the blood volume. The acacia is determined as furfurol-phloroglucid, according to the method of Kröber.

The blood volume changes in shock and the modification of these by acacia. H. S. GASSER, W. J. MEEK and J. ERLANGER.

The volume of the blood was determined by the acacia method. The erythrocytes were also counted as an index to the plasma changes. The forms of shock studied were those produced by exposure of the intestines, injection of adrenalin, and temporary partial occlusion of the vena cava or the aorta. The findings, in all cases were essentially the same. There was a decrease of the plasma amounting to an

average of 22.2 per cent of the total blood volume. In the cases where the acacia was injected this decrease was strikingly less being on the average only 7.4 per cent of the normal.

Loss of plasma accounted for all the decrease in blood volume in one-third of the cases. Where the decrease in blood volume (acacia determination) was somewhat greater than that calculated from the red cell count the difference could be explained by haemorrhages into the tissues. These were a constant finding in the intestine, spleen and other organs. In a remaining group the blood volume decrease was much greater than that indicated by the red blood cell count. This difference could only be explained on the assumption of regions of stasis.

The filtration of the plasma might be due to decrease in the colloidal osmotic pressure of the plasma, rise of arterial blood pressure or increase in the permeability of the vessels. The first possibility may be at once discarded. The high blood pressure in adrenalin shock causes filtration, but the fluid returns when the pressure falls and polycythaemia only remains permanent when the decreased supply to the tissues has resulted in damage. When the cava is clamped the venous pressure is high but the arterial pressure is so low that there is little chance for filtration, other factors being equal. When the aorta is clamped neither the venous nor arterial pressures are high in the posterior part of the animal, but filtration is both a constant and marked phenomenon. One must turn therefore to decreased permeability of the vessels to explain the phenomena.

The acacia exerts its conserving influence on the plasma under conditions which are highly pathological, under conditions in which normal plasma leaves the vessels not only without a rise of arterial pressure but even when the pressure is low. Bayliss has suggested the use of acacia in the rendering of a solution isosmotic to plasma to aid its retention in the vessels when injected intravenously. In our experiments where normal plasma leaves the vessels acacia must have a further influence. In the amounts used the colloidal osmotic pressure of the plasma would be increased about 13.5 per cent. This would act in the direction of decreasing filtration and increasing absorption. Our blood counts, however, have given us no evidence that any appreciable expansion of the plasma volume from the tissues takes place in one hour. The possibility that acacia acts as a calcium salt in decreasing the permeability of the vessels is worthy of consideration.

The effects of injecting acacia. WALTER J. MEEK and HERBERT S. GASSER.

In view of the use of acacia in perfusion solutions a series of experiments has been made to determine its effects on experimental animals. Injections of large amounts of 20 per cent acacia have practically no effect on blood pressure other than the mechanical one of increasing the blood volume. The heart rate is not influenced other than that due to the slightly raised blood pressure. After hemorrhage acacia seems

to maintain blood pressure better than salt solutions. Respiration is unaffected. The urinary secretion after acacia can be stimulated by sodium nitrate apparently as well as normally. An animal under ether may have acacia injected until the blood is a 10 per cent solution with no symptoms of disturbance. Intact animals have had their blood made up to 4 per cent acacia with no unfavorable symptoms. Acacia leaves the blood stream in the early stages at the rate not exceeding 10 per cent per hour. This rate soon slows and fairly large amounts of acacia may be found in the blood two days later. A pentose reaction may be obtained from the urine an hour after the injection of acacia.

Diet experiments bearing on carbohydrate luxus-consumption and wasteful eating. ADDISON GULICK.

The purpose of the tests was to determine whether excessive consumption of carbohydrate by a person of the characteristically lean physical type did not stimulate the organism to oxidize away a large part of the excess supply of fuel.

These experiments covered one and three-fourths years of more or less controlled eating with 330 days on strictly measured experimental diet. Diet in the strict periods was 90 to 675 grams per day of cereal foods (air dry) plus 3 pints milk, usually about 100 grams egg, and 0 to 50 grams butter.

At the start and finish, minimum food requirements were determined. Between these periods there was prolonged over feeding up to a maximum of 4100 calories and then a return to low weight by restricting the diet to about 1800 calories for six weeks. Activities were held as nearly uniform as possible.

Conclusions. 1. Minimum food requirements were somewhat greater than would be expected from the activities. Initial test: Slight weight loss during four weeks on 2750 calories, body weight about 62 kgm., sleep 8.2 to 8.5 hours per night, daily walking 5 miles or less. Final test: Weight constant twenty days on 3200 calories, body weight 61.5 kgm., average sleep 8.3 hours, daily walking 4.9 miles, bicycle 4.7 miles.

2. Under non-experimental conditions, the regulating influence of the sense of appetite and of satiety is an important factor in holding the weight at its rather constant level of 63 to 66 kgm. For upon eating persistently more than was relished (3600–4100 calories), the weight was gradually raised to 74.7 kgm. This conclusion probably has wide application, as the man experimented upon was believed, at the start, to show the very reverse condition.

3. If there is a luxus factor it is not overwhelmingly large. Metabolism was high in the periods of heavy feeding, but not high enough to give good proof of a true luxus oxidation, over and above the specific dynamic effect of the food, and the added fuel cost of activity of an organism enlarged by fattening.

4. If a luxus oxidation occurs at all, its effect is ended within 14 hours after taking food. Evidence is from the moderate or almost low post-

absorptive basal gas metabolism while on a 4000 calory diet. Gas exchange = 71.5 calories per hour, = 0.97 calories per kilogram, = 34.4 calories per square meter. (Determined by courtesy of the Carnegie Nutrition Laboratory in a Benedict bed respiration chamber.)

5. From an economic standpoint, the increased fuel expenditure found in these experiments with a high diet and moderate activities, was a pure waste.

Tests of methods of control of the clothes louse. WM. MOORE.

Sachets are not successful.

Talc 20 grams, creosote 1 cc., sulphur 0.5 gram is six times as effective a louse powder as NCI, causes less irritation to the skin and is dry, hence easier to apply.

Impregnation of the underwear is not possible, but a cheese cloth suit impregnated with a saturated solution of sulphur in creosote could be successfully worn outside of the underwear.

Chlorpicrin can be used as a fumigant, penetrating the clothing and killing the lice in all parts of the clothing in fifteen minutes and the eggs in thirty minutes. By increasing the heat in the fumigation chamber the time required to kill the eggs could be reduced.

The tension of the blood gases in the blood entering and leaving the lungs. R. G. PEARCE and A. NICHOLSON.

We believe that our data indicate that so long as the minute volume of the circulation and the respiration increase directly with the metabolism, the difference between the tensions of the gases in the blood entering and leaving the lungs remains approximately constant. However, during acute exercise when the volume rate of the circulation fails to keep pace with the rate of increase in the circulation, the difference between the tension of the gases in the blood entering and leaving the lungs progressively increases, and the minute volume of the respiration is increased out of proportion to the degree of the oxygen intake or carbon dioxide output. The progressive increase in the difference in the tension of the gases entering and leaving the lungs is brought about by the hyperpnoea which reduces the tension of carbon dioxide in the alveolar air below that normally present or expected, and to the very rapid and disproportionate increase in the tension of the carbon dioxide in the blood entering the lungs.

The cause of the hyperpnoea in acute violent exercise is questionable. The failure to find a diminution in the alkali reserve of the body argues against the accumulation of organic acids from incomplete oxidation, and this together with the low tension of the carbon dioxide in the alveolar air speaks against the increase being due to stimulation of the center by increased acidity or carbon dioxide hormone. The possibility of oxygen want stimulating the center directly, or indirectly, through afferent respiratory fibers in muscle nerves is suggested by the high respiratory quotient found at higher levels of exercise. An inadequate minute volume of the blood flowing through the center during exercise

because of venous engorgement due to right heart insufficiency might explain the phenomenon.

The methods employed for the estimation of the tension of the respiratory gases in the blood entering and leaving the lungs at various levels of metabolism afford a means of judging the heart's reaction to and capacity for acute work.

Reflexes and clonus recorded graphically. R. Edwin Morris and L. G. Rowntree.

Hitherto the physician has been compelled to depend upon memory pictures when recalling reflexes and clonus. Obviously, this is unsatisfactory. It was with the idea of meeting a glaring need that an attempt has been made to devise methods of graphically recording these phenomena.[1]

Two great problems are encountered: (1) methods of record, and (2) interpretation of records. Our efforts up to date have centered on the former. In the beginning difficulty was experienced in securing consistent records, but, after a year's experience, various difficulties having been met one after another, we finally feel confident that consistent records of reflexes can be obtained. At present we are considering their interpretation and are meeting with some degree of success. However, as many of the records are relatively complex the question of interpretation will be deferred and will be the subject of further investigation and of future publication.

The electrocardiograph apparatus of the Taylor Cambridge type has been utilized, additions being made to meet our particular problems. The knee-kick and ankle clonus have been the special phenomena under investigation.

In securing records of knee-jerks, the patient is seated in a specially devised chair upon an electrode molded to the form of the gluteal region. The chair is sufficiently high from the ground to permit the feet to swing free. Mechanical leg attachments, in the form of laterally adjustable swing boards, are hinged to the front edge of the chair. These are adjusted to the patient's legs and secured to them by means of straps. Electrodes are attached, but only one at a time is connected with the string galvanometer. Preliminary study is made and the point yielding maximum resistance is marked. This constitutes the invariable point of contact in eliciting the reflexes. An insulated contact, and a small wire net attached by adjustable supports to the swing board, are placed over the area already marked. The hammer which produces an electric contact resulting in a fling in the signal magnet, is adjusted to the horizontal bar so that it strikes directly in the desired spot on either leg. Along the handle of the hammer there are five holes. The hammer is usually secured on the horizontal bar through hole (1), thereby fur-

[1] The credit for the mechanical devices utilized is due to Doctor Morris. After writing up our work—our attention was called to the work of Bornstein and Saenger (Deutsche Zt. f. Nervenheilkunde, 1914, Bd. 52), who also utilized the electrocardiograph in this connection.

nishing the lightest stroke possible with this apparatus. The second lighter horizontal set-bar is so placed that the hammer may be dropped from any desired angle. Ordinarily, however, 90 degrees is the one advised. In the other edge of the swing board are inserted three eyelets, to one of which is attached a hook which is secured to an inelastic cord which runs over an adjustable frictionless pulley to a hanging indicator suspended in front of the aperture in front of the lens of the electrocardiograph. The indicator produces a shadow parallel to that of the string galvanometer. Holding the indicator in position on the other side is a coiled steel spring, the tension of which is adjustable. So long as the indicator remains in a vertical position the tension on the string leading to the swing board is constant. A time-marker indicating one-tenth of a second has been used in most of the work.

In these records the upper tracing represents the time-marker, one tenth of a second being indicated. The second record represents the electrical response, the picture resulting from the shadow of the galvanometer string. The third record represents the mechanical response as pictured by the movement of the indicator; and the fourth depicts the instant of contact. In many of the records the deflection resulting from the introduction of a millivolt is shown just prior to the reflex. This indicates the tension of the string.

The cerebral center of mastication. F. R. MILLER.

Ferrier, Mann and others observed that typical movements of mastication could be evoked in the rabbit by stimulation of the cerebral cortex a slight distance in front of the Sylvian fissure. The present research was undertaken with the idea of analysing more completely the cerebral mechanisms concerned in these movements.

By the procedure of dividing the lower jaw at the symphysis it was determined that stimulation of the cortical area of one side causes synchronous chewing movements executed by both halves of the jaw. It was also found possible to elicit similar bilateral chewing movements from the subcortical tracts as far back, approximately, as the commencement of the crus cerebri. Farther back than this only continuous jaw closure was obtained; this appeared to be mainly ipsilateral.

An endeavor was made to localise the subcortical centre of mastication. Réthi considered it to be within or below the thalamus and above the crus. A transverse section was made across both cerebral hemispheres at a distance of 16 to 17 mm. behind the anterior zygomatic angle. Stimulation of the appropriate point on the cross-section of each hemisphere yielded chewing and swallowing. A slice a few millimeters in thickness was next removed from the left hemisphere. Stimulation applied to the left section evoked now usually only continuous contraction of the jaw muscles apparently chiefly ipsilateral. Stimulation of the original point in the right section still elicited chewing and swallowing.

It is evident, therefore, that the masticatory centre is situated between the section of the right hemisphere, which passes through the corpora

mammillaria and the habenular nuclei, and the level of the section of the left hemisphere, which, in a number of experiments, passes through the medial geniculate bodies and the posterior commissure.

The point yielding mastication was localised on the more anterior cross-section by unipolar stimulation and was found to correspond with the medial portion of the pes pedunculi.

The relation of lesions of the optic thalamus of the pigeon to body temperature, nystagmus and spinal reflexes. FRED T. ROGERS.

As was long ago recognized by many early workers on the brain of the pigeon, the effects of decerebration vary according to whether or not the thalamus is also injured. Lesions of the thalamus are followed by a long continued flattening of the feathers against the body (Bechterew). Decerebration without thalamic lesion does not cause this change in the position of the feathers. Intra-peritoneal injection of pilocarpine in normal and in decerebrate pigeons causes a similar flattening of the feathers. The body temperature of decerebrate pigeons with thalamic lesions varies with the temperature of the cage. The temperature of decerebrate pigeons without thalamic lesions remains normal, but injection of pilocarpine in these birds is followed by changes in body temperature varying according to the temperature of the environment. Injection of pilocarpine sufficient to flatten the feathers of a normal pigeon produces very slight temperature changes (1 to 2°C.).

In decerebrate pigeons with *lesions* of the thalamus, the behavior varies with the body temperature. Depression of body temperature to 25° to 30° C. is followed by diminished reflex response to irritating vapors to the nostrils: to the puckering reflexes of the cloaca, and to the stimuli of hunger.

Complete decerebration leaving the thalamus intact does not lead to the disappearance of the nystagmus of head and eyes (Ewald). If the thalamus also be removed, whether or not the nystagmus disappears depends on the body temperature. If the temperature be lowered to about 30°C. the quick component disappears and the deviation (on rotation) persists. If lowered to 25°C. the deviation also disappears. If body temperature be brought back to 39°C. both deviation and quick component reappear. Involvement of the oculomotor nuclei causes disappearance of the eye nystagmus. In lowering the body temperature the head nystagmus persists after the eye nystagmus has ceased.

On the stimulation of the vagogastric medullary centers by drugs. FRED T. ROGERS.

In the turtle with the spinal cord sectioned at the level of the third cervical vertebra but the circulation through the head intact and the vagi normal, the injection of 0.5 cc. to 1 cc. of a 1:1000 solution of picrotoxin in Ringer's solution into the carotid artery leads to a powerful tetanic contraction of the stomach. This effect does not follow the injection if both vagi have previously been sectioned. Electric stimulation of the floor of the fourth ventricle causes a similar contraction.

This contraction is followed by a prolonged refractory stage of the gastric musculature to vagus stimulation.

In the dog, with splanchnic nerves previously sectioned, the injection of apomorphine in doses too small to cause vomiting leads to a marked diminution in the tonus of the stomach and to a cessation of peristalsis, so far as can be determined by the balloon method of recording gastric contractions. Vomiting caused by apomorphine is not preceded by any gastric contractions so far as this method will indicate. Picrotoxin caused the same changes in the gastric contractions as did apomorphine.

Comparison of the rhythm of the respiratory center and trapped wave in cassiopea. J. F. McClendon.

Mayer has shown that if a nerve impulse is started in a ring of nerve tissue of the jelly fish, cassiopea, it will pass round this ring at a uniform rate and can be tapped off at one point as a rhythmical impulse. The nerve tissue in the ring is in the form of a network with numerous synapses. I have shown that the rate of the nerve impulse per centimeter is not affected by stretching the ring and, therefore, the number of impulses per second that may be tapped off at one point in the ring may be varied by stretching the ring. The rate of the nerve impulse is affected, however, by additional stimuli applied to the ring or to nerve fibers coming into the ring. These additional stimuli retard the rate of the nerve impulse.

If we imagine the ring to be the respiratory center and that nerves are stimulated by the distention of the lungs and carry stimuli to the ring thus slowing the rate in the ring, the rate of breathing will be retarded. In this way we may make a model of the respiratory center out of very simple nerve tissue. Since the rate of the nerve impulse in the ring can be changed by altering several factors the rate of respiration would depend also on these.

Some points in the nervous regulation of respiration in the cat. C. C. Gault and F. H. Scott.

In these experiments respiration was recorded by recording the movement of the diaphragm by means of a spoon inserted between the liver and diaphragm (Rosenthal's method). Section of the vagi leads to the ordinary effects observed in other animals (prolonged inspiration and slow respiration). If in such an animal the cord be divided in the lower cervical region this type of respiration disappears and is replaced by one in which the ratio of expiration to inspiration is practically normal, although expiration and inspiration are prolonged. In a normal animal section of the cord leaving the vagi intact produces a respiration with prolonged expiratory phase. This same type of respiration is produced by section of the posterior roots of the thoracic nerves. It is evident that the impulses which come from the muscles and joints of the thoracic wall produce an opposite effect on the respiratory center from those coming from the lungs.

The effect of alterations of blood pressure on the blood of the rabbit. F. H. SCOTT.

Lamson reported that adrenalin did not produce a polycythemia in rabbits while it did in other animals. I find rabbits respond like other animals to alterations of blood pressure. A rise of pressure in the rabbit leads to an increase in the haemoglobin content of the blood and a decrease of pressure to a decrease of the haemoglobin content. Rabbits are extremely sensitive to the effects of small haemorrhage. The loss of a few cc. of blood is followed by a dilution of the blood. However, I believe Lamson's results are due to waiting till the blood pressure had returned to normal or sub-normal or to not getting an alteration in blood pressure.

Location of adrenalin vasodilator mechanisms. FRANK A. HARTMAN.

Destruction of the brain does not interfere with the adrenalin vasodilator mechanism for the intestine. Pithing of the cord in the thoracic region often decreased the amount of intestinal dilatation from adrenalin. However pithing of the whole cord did not completely destroy this reaction of the intestine.

Loops of intestine perfused with Ringer's solution, but with intact nervous connections, dilated when adrenalin was injected into the external jugular, even though all splanchnic fibres were cut. Occasionally the dilatation was preceded by slight constriction. It seems from this that adrenalin produces dilatation of the intestine by stimulation of the sympathetic ganglia supplying them.

The adrenalin vasodilator mechanism for the hind limb must be below the thoracic cord because destruction of the whole central nervous system that far down does not prevent its action.

Adrenalin vasodilator mechanisms in the cat at different ages. FRANK A. HARTMAN.

Adrenalin fails to produce a fall in blood pressure in kittens less than about eleven weeks old. The depressor effect at this age is small and may be inconstant. In fact, the increasing of the depressor effects from the slight fall succeeding a rise in younger animals to a marked almost pure fall in adults indicates a gradual development of the adrenalin vasodilator mechanism. This fall in blood pressure seems to be due to vasodilatation in skeletal muscle, for the two begin to appear simultaneously.

In three kittens from nine to eleven weeks of age both limb and intestinal adrenalin vasodilator mechanisms were sought. All three gave active limb dilatation, but no intestinal dilatation. It seems therefore that the mechanism for the intestine develops later. This supports the view that the two mechanisms are of different types.

Vasodilator nerves of the skin. H. RICHARDSON and O. WYATT.

Bayliss showed that stimulation of the posterior roots leads to a vasodilation of the vessels of the part. The work of Bruce rendered it very

probable that this was a case of axone reflex. Bruce used the inflammatory process as the basis for his work. We have followed the changes using a plethysmograph. If the paw of an animal be placed in a plethysmograph a vasodilation (increase volume of the leg) may be obtained by slow rhythmic stimulation of the peripheral ends of the cut cutaneous nerves outside the plethysmograph or by the same stimulus applied to the skin inside the plethysmograph. If cocaine be applied to the skin it is still possible to get a vasodilation from the nerve but none when the stimulus is applied to the skin.

A note on the mechanism of heart muscle contraction. MONTROSE T. BURROWS.

In this paper two facts were emphasized, the first is that tissue cells within a tissue culture cannot subsist upon the food material found within a medium of blood plasma. They grow also in a medium of salt solution. The cells that grow in a tissue culture are those at the periphery of the fragment. They obtain their nutriment from the cells that disintegrate in the center of the tissue fragment. The material use for food diffuses out over the surface of the medium. It is colloidal in nature and insoluble in the medium.

The second fact is that foetal heart muscle cells are essentially fluid in nature. The peculiar mechanical organizations essential for growth, migratory movement, rhythmical contraction or other forms of activity are differential surface tension phenomena established and controlled by the organization of the environment. By changing the mechanical organization of the environment one may change a contracting heart muscle cell to one which grows and divides by mitosis and is indistinguishable from a sarcoma cell. The growing and dividing cells are those cells which lie at the interval between the substances diffusing from the fragment over the surface of the medium and the medium itself. The contracting cells are elongated, cylindrical-shaped cells stretched through the liquid medium between a surface similar to that suitable for growth and the ends of tense bands of fibrin. The end of the cell in contact with the tense bands of fibrin is in metabolic equilibrium. Heart muscle cells completely embedded in a mass of fibrin show no metabolic activity in the presence of food and oxygen. They have been kept in this position in the incubator for six months without disintegrating. They grow again when removed to a suitable environment. The other end of the contracting cell is in contact with a surface similar to that upon which cells grow. The curve of growth of heart muscle cells has been studied and it has been found to follow the law of mass action and the cells come to an equilibrium before food and oxygen is exhausted. This inactivity or equilibrium is not disturbed by washing with serum but only when colloids (fibrin) or dead cells are added. It is assumed to be due, therefore, to the accumulation of waste product insoluble in serum but soluble in colloidal substances. This product in the presence of the surface food layer decreases the surface tension of the cell. With one end of the cell in equilibrium, the other yielding an

insoluble product it is evident that periodically this insoluble product may be broken up and an electric current pass (Bredig's phenomenon). It has been shown that oxygen is absorbed only during the relaxation period, tension is then developed. Lactic acid is liberated at contraction. The author has found that lactic acid causes an increase in surface tension.

Thus a theory for the mechanism of heart muscle contraction has been developed which explains the energy transformations and the physicochemical changes known to occur in heart muscle contraction.

The experiments were made with single and completely isolated rhythmically contracting heart muscle cells.[1]

Effects of external temperature and certain drugs on thyroid activity. C. A. MILLS.

Effects of variations of external temperature on dogs, cats, guinea pigs, and rabbits were studied. It was found that animals kept at 30 to 37°C. for several days showed the following changes in almost every case: the colloid content of the vesicles was increased in amount, presented a uniform appearance, and stained rather intensely with eosin; the epithelial cells lining the vesicles were decreased in height, often entirely flattened, their cytoplasm and nuclei appearing rather compact and dense.

Animals subjected to low temperature, such as out-door winter temperature, for the same length of time exhibited a markedly different set of histological changes in the thyroid. The colloid decreased in amount, sometimes disappearing almost entirely, stained less intensely with eosin, and contained vacuoles of various sizes around the edge near the cells. These vacuoles, in some cases, entirely replaced the colloid, or left only shreds of it, looking as if the vacuoles were formed by the resorption of the colloid by the cells, leaving in its place a clear nonstaining fluid. The epithelial cells lining the vesicles became elongated to cuboidal or columnar types, both cytoplasm and nuclei apparently having enlarged and become less dense.

If we take as the index to the activity of the thyroid during the period of observation, the increase or decrease in the amount of stored colloid, its staining reaction, and the presence or absence in it of vacuoles, and also the character of the cells lining the vesicles, then the thyroid is shown to respond to temperature variations, other conditions being kept as near constant as possible. This same work is now being tried on opossums, using Bensley's stain to demonstrate the amount of true secretion antecedent, as he terms it in his work, found in the epithelial cells under these different conditions, in order to see if the two methods of gauging thyroid activity yield comparable results.

Certain drugs were also tried, and the histological changes in the thyroid noted. Morphine, injected into cats in amounts sufficient to produce hyperexcitability, caused changes in the thyroid closely resembling

[1] Burrows: Münchener Med. Woch., 1912.

those described above as resulting from low temperatures. On rabbits the effects were identical with those of high temperatures. Quinine had the same effects on the thyroid in rabbits as did morphine. Strychnine is now being tried to see if the activity of the gland is increased along with the hyperexcitability of the animal.

The influence of pituitary extracts on the daily output of urine. H. M. REES.

The earlier work on the extract from the posterior lobe lists it as a diuretic, while recent investigators conclude that it is an antidiuretic.

It was our purpose in this investigation to find out: (1) whether the subcutaneous injection of pituitary extract will cause any quantitative variation in the daily output of urine; (2) whether such injection will in any way affect the quantity of urine excreted, and, if so, to find out if possible the factors involved.

Cats and rabbits were used as the experimental animals. The observations in each case were over three to ten days for the control and three to ten days for the injection.

The daily quantity and the specific gravity were the principal points noted. Variations in rate of output were also noted in several of the experiments.

It was found that pituitary extract does not, when injected subcutaneously, alter the amount of urine excreted per day in cats and rabbits, nor does it cause any marked variation in the specific gravity of the urine. There is, however, a very striking effect on the rate of excretion. Subcutaneous injection of pituitary extract causes a delay of seven to eight hours before the beginning of the diuresis which follows the injection of the large amounts (150–200 cc.) of water.

Evidence of toxic action of ovaries of gar. CHARLES W. GREENE, ERWIN E. NELSON and EDGAR D. BASKETT.

The reputed toxicity of gar ovaries was tested by feeding fresh ovaries to chickens, white rats, cats, and dogs. Chickens were fed from 5 to 75 grams each in amounts distributed over several feedings. These tests were run at intervals on individuals of pens under observation from thirty to fifty days. Control chickens were fed fresh gar meat and carp ovaries in addition to grain and kitchen scraps. This diet was also given to experimental individuals between tests. All ovaries and meats were fed fresh, generally within an hour after the fish had been killed. The symptoms produced were loss of tone and paralysis of the crop, loss of appetite, diarrhoea, loss of weight, muscular weakness, disturbance of the circulation as shown in the comb and wattles and depression of the central nervous system. If large amounts of the gar ovaries were fed the chickens became gradually weaker, dying after three or four days. If the ovary was withheld, in time the chickens slowly recovered. The ovaries are taken freely the first feeding, but never voluntarily the second time, hence forced feeding was used. Single feeds of 5 grams was the minimum toxic dose producing just perceptible symptoms.

White rats died after eating quantities as small as 5 grams of gar ovary. The toxic effects were general malaise, diarrhoea, marked diureses, loss of appetite, muscular weakness, and death. Autopsy showed stomach and intestines dilated by gas and usually with a severe congestion of the ileum.

Cats were given from 5 to 35 grams in a single feed. This was taken voluntarily the first time but never the second. Fresh ovaries and ovaries cooked by steam produced the same effects. In all tests the ovaries were vomited within two and a half hours, usually earlier. Occasionally there was a slight diarrhoea but no other symptoms were noted. The same effects followed when the ovaries were fed to dogs. Violent vomiting was exhibited. The last vomited material was partly digested Organ tests are under way and chemical separations likewise. The latter indicate that the toxic substance lies in the globulin fraction though we do not consider this point fully established.

Some electrical phenomena of the submaxillary gland. ROBERT GESELL.

The electrical variations of the submaxillary gland activated in various ways were graphically recorded. Both two gland leads and single gland leads were employed.

To interpret the deflections, blood pressure, secretion, and volume flow of blood were simultaneously recorded.

To maintain a constant condition of the animal the volume flow of blood was studied by an automatic and bloodless method especially devised for these experiments.

The electrical deflection obtained from prolonged chorda stimulation, with the usual lead, commonly shows four negative waves.

The deflection obtained by chorda stimulation, with the general condition of the animal remaining constant, is variable depending largely on four factors—duration of stimulation, strength of stimulation, duration of the period of rest, and the position of the electrodes on the gland.

By keeping all four of these factors constant, provided the period of rest is sufficient, superimpossible deflections can be obtained. This suggests the practicability of studying glandular processes by the electrical method, and permits the establishment of controls for studying the effects of introduction of other variables.

Results of some experiments indicate that contractility of the salivary ducts may account for the first negative wave. Other causes such as relaxation of the blood vessels were not ruled out, however.

The second and third negative waves have much in common. In most experiments the amplitude of these waves varied roughly with the rate of secretion. This correspondence may in part account for the dip in the deflection between the second and third waves elicited by prolonged chorda stimulation. Certain experiments, however, indicate that change in rate of secretion may not be the sole cause of this dip.

Frictional electricity as produced by flow of saliva or blood through the vessels is probably a minor factor in determining the deflection.

Obstruction of either the salivary duct or the carotid artery during glandular activity, however, markedly affects the electrical variation.

The effect of arterial obstruction is probably produced by the regulating effect of blood supply on glandular metabolism.

The fourth negative wave is not constant. When it does occur, it may last as long as twenty minutes, and may possibly represent the progress of recovery processes.

There seems to be a number of factors operating to produce the resultant deflections. Interpretations of the deflections are, at present, only tentative.

An automatic and bloodless method of recording the volume flow of blood. ROBERT GESELL.

The device used in this method consists of an electrical arrangement which permits the automatic filling and emptying of a segment of vein draining the tissue under study.

The apparatus described was devised primarily to record the volume flow of blood through the submaxillary gland, but it can be used for other tissues as well.

In measuring the volume flow of blood through the submaxillary gland, all the veins emptying into the external jugular vein, with the exception of those coming from the gland, are ligated. The jugular vein is then placed in a trough under an emptying plate and a cut off. The cut off is held down on the vein by the pull of a spring, preventing the flow of blood to the heart. In consequence, the blood accumulates in the vein below the emptying plate, raising this plate until an electrical contact is made, which simultaneously opens the cut off and presses down the emptying plate. The emptying of the vein breaks the contact, the cut off closes, and the process repeats.

The rate of filling and emptying varies directly with the volume flow of blood. This method can be made quantitative by calibration of the vein.

Vagotonic and sympathetic-atonic effects on gastric motility. T. L. PATTERSON.

The studies were made upon the bullfrog, Rana catesbiana, and the balloon method was used. All the animals were stomostomized[1] and normal records of the gastric hunger contractions with acid inhibtion obtained, 5 cc of 0.5 per cent hydrochloric acid being used in each case. They then underwent a second operation in which either both vagi or both splanchnics were sectioned and the above observations repeated. The vagi were cut in the region of the neck; the splanchnics in the region of the coeliac plexus after laparotomy.

Section of both vagi with the splanchnics intact leads to a sympathetic-atonic condition of the stomach with about the normal type of hunger contractions persisting, with the exception that, on the whole,

[1] Patterson: This Journal, 1916, xlii, 61.

they appear to be of a slower rate and slightly weaker, whereas the inhibition produced by the acid is quicker and more marked than in the normal animal. Section of both splanchnics with the vagi intact leads to a hypertonic stomach. The contractions are small tending to run into incomplete tetanus with an increased rate, while the acid inhibition is almost without effect there being only a very slight decrease in the height of the contractions.

Likewise, the same general influence which these two sets of nerves exert separately on the gastric apparatus may be shown when the splanchnitized stomach is superimposed upon the vagotomized stomach from two frogs of equal size. The latter or larger stomach represents the atonic and the former or smaller the hypertonic while the normal stomach takes an intermediate position between the two. It may be said, therefore, that the reciprocal or contrary innervation of Meltzer which may be termed antagonistic tonus, may be physiological as long as it serves the purposes of the organ in question in a beneficial manner. It is pathological as soon as the tonus of one or the other is so exaggerated that the common welfare of the organ is in danger and that is exactly what happens in the splanchnitized frog's stomach where the hypertonus of the vagus leads to a state of overexcitability, or to the Eppinger-Hess condition of vagotonia. Further investigations on this subject are in progress.

Studies on gastric secretion in man and dog: Gastric secretion and urine ammonia. A. C. Ivy.

This study consisted in the examination of the urine ammonia and gastric juice during (1) gastric stimulation followed by absorption in the intestine, (2) gastric stimulation without absorption in the intestine, (3) intravenous injection of water, (4) the absorption from the intestine of water, acid and alkali introduced by duodenal tube and duodenal fistula, and (5) during diuresis.

Gastric analyses were made every fifteen minutes. Urine was collected in fifteen or thirty minute intervals by catheter in dogs and voluntary micturition in man. Controls were made for one-half to one hour preceding the experiment to determine the continuous gastric secretion and urine ammonia. Conclusions are based on from three to ten trials of the same experiment in each individual. The work has been done on five normal men (the injections via duodenal tube were done only on one man), and has been repeated on female dogs with gastrostomy, duodenostomy and perineorrhaphy (perinaeum slit to expose urethral orifice). For the determination of urine ammonia the Folin macrochemical method and Folin-Nessler method, with the permutit modification[1] have been used.

It has been found that:

1. There is an increase in urine ammonia beginning one-half to one hour after the ingestion of a meal. This increase varies in the same individual and in different individuals on a constant diet.

[1] Folin and Bell: Journ. Biol. Chem., 1917, xxix, 329.

2. During gastric stimulation by food or water followed by absorption in the intestine there is an increase in urine ammonia.

3. During gastric stimulation by food or water *not* followed by absorption in the intestine no increase in urine ammonia results.

4. Intravenous injection of 200 cc. of water causes some gastric stimulation without an increase in urine ammonia or urine output.

5. (a) The absorption of water from the intestine causes some diuresis but no change in urine ammonia.

(b) The absorption of acid from the intestine causes some diuresis with an increase in urine ammonia.

(c) The absorption of alkali from the intestine causes diuresis with a decided decrease in urine ammonia.

6. Diuresis per se causes no change in urine ammonia.

So gastric secretion and urine ammonia are related in that the urine ammonia is increased by the absorption from the intestine of the acid product of the gastric secretion.

During the course of this study it has also been found that:

1. Copious water (300 cc. in Pavlov dogs and 500 cc. in man) with the meals causes an increase in the amount and in the free and total acidity of the gastric juice. There is no change in peptic activity.

2. The latent period of the gastric glands of man when stimulated by water is from five to seven minutes.

3. All stomachs are not stimulated by water, which seems to depend upon the rate of emptying the water, e.g., those stomachs that empty water slowly (less than 150 cc. in fifteen minutes when 400 cc. are drunk) respond much more than those that empty water fast.

The effect of water and sodium bicarbonate on gastric secretion. C. E. KING and W. W. HANFORD.

Dogs with the miniature stomach according to Pavlov were used. A uniform diet was maintained. The juice secreted after the introduction of distilled water was taken as the standard, and with it was compared the juice secreted after the administration of a 1 per cent solution of sodium bicarbonate. Experiments were carried out in which the water and alkali were introduced into the empty stomach, also with the meals and during the various stages of digestion. Dogs weighing about 15 kilos were used. The volume of liquid given each time was 400 cc. The following is a summary of the observations and conclusions:

1. Water excites the flow of gastric juice.

2. Water starved dogs secrete no appetite juice when shown water in the container from which they are accustomed to drink, or when placed near running water.

3. The acidity of the gastric juice runs parallel with the rate of secretion until a maximum is reached. This maximum varies in different dogs but is near 0.5 per cent HCl.

4. Water given with meals or during digestion results during the following hour in an increase in the amount of juice secreted over that which would be secreted on the administration of either water or meat alone. This also holds true for 1 per cent sodium bicarbonate.

5. One per cent sodium bicarbonate excites the flow of gastric juice, being somewhat less effective than distilled water. The depression is not so marked as reported by Pavlov.

6. The acidity of alkali juice, when the alkali is introduced into the empty stomach, is on an average a little lower than that of water juice, but not materially different when given with meals or during digestion. This refers to total acidity. The combined acidity of alkali juice is a little higher than that of water juice.

7. Water and alkali juices possess approximately the same peptic activity, but both are much less active than meat juice.

8. No injurious effects were noted on the continued administration of 1 per cent sodium bicarbonate.

The effect of water on gastric secretion. GEO. F. SUTHERLAND.

This is a study of the mechanism of the stimulating action of water on gastric secretion. Psychic secretion from the drinking of water was not tested, since its importance has already been shown by Carlson, Orr and Brinkman. Two other factors were studied, (a) dilution of the blood by intravenous infusion, and (b) more rapid absorption of secretagogues.

The intravenous infusion of 10 to 20 cc. of distilled water per kilo body weight in dogs with a Heidenhain or Pavlov pouch, or a simple gastric fistula, is stimulant to gastric secretion. Ringer's solution variously diluted, or a 5 per cent gelatin solution in Ringer's produced a similar response. Water introduced into the small intestine without the possibility of regurgitation into the stomach produces a slight though definite response. Introduction of water by stomach tube stimulates gastric secretion in man and dogs. Water introduced into the gastro-intestinal tract has a greater stimulating effect than by intravenous infusion probably because it increases the absorption of secretagogues.

A 0.23 per cent calcium chloride solution (16 cc. per kilo body weight by intravenous infusion) did not inhibit the secretion caused by gastric secretin.

There is a periodicity in the gastric secretion in starvation, periods of low activity alternating with periods of "spontaneous" increased activity.

In pups and kittens, the gastric glands are not able to secrete free HCl until about the time of birth. In guinea pigs, this power is developed earlier.

Further studies of the mechanism of the clinical measurement of blood pressure. ALBERT M. BLEILE and CLYDE BROOKS.

The demonstration of the physics of the arm band indirect method of measurement of blood pressure by Brooks and Luckhardt[1] opened

[1] Brooks and Luckhardt: Demonstration before the American Physiological Society, December, 1 14, also, The chief mechanisms concerned in clinical methods of measu ing blood pressure, this Journal, 1916, xl, 49.

and paved the way to a more complete understanding of the pulse sounds in the auditory method of clinical measurement of blood pressure.

The present work consists of a close observation of the pulse sounds in a large number of cases and an attempt to correlate these with what has just been discovered regarding the behavior of the blood vessel during the application of the arm band method.

The result indicates that the sounds heard are more varied and complicated than are generally recognized. There are certainly more than the four phases described by Korotkow. They seem too many and too complicated to describe here.

However it may be definitely stated that the "swish sounds" occur at such a time and in such a manner as to be in agreement with the hypothesis that they are due to the squirting of blood through the narrow orifice of the flattened but incompletely closed blood vessel.

A full report of these studies will be published shortly.

The length of the systole and the diastole of the human heart. WARREN P. LOMBARD and OTIS M. COPE.

There is great and immediate need of practical methods of determining the functional condition of the heart muscle, and of the irritability of the nervous mechanisms which regulate its action. Ordinary muscles when fatigued or degenerated show a longer latent period and a more prolonged contraction than normal, and presumably the same would be true of the heart muscle. Accurate measurement of a sufficiently long series of systoles and diastoles would give information not only concerning the heart muscle, but concerning the behavior of the respiratory and vaso-motor nervous mechanisms which influence the heart rate. There ought to be a table which would supply at a glance the average length of the systole and diastole of the normal heart, and the ordinary variations from the average, by every ordinary heart rate, the rate being estimated from the length of the individual cycles. The writers have begun the preparation of such a table. Although photographic records of the heart sounds would probably supply the most accurate measurements, the writers have thus far employed tambour records of the carotid pulse, because, if these proved sufficiently reliable, they would be of more practical use. The results of the measurement of the systoles and diastoles of 1600 heart cycles of twenty normal young men were shown in a chart. The chart gave for every heart rate from 60 to 120, as calculated from the cycles, the average length of the systoles and diastoles, the variations from these averages, and the number of cycles and of men supplying the data for each heart rate. Another chart illustrated the great variations in the lengths of the systoles and diastoles which may normally occur during one minute, as a result of inspiratory and vaso-motor effects.

A new criterion for the determination of the diastolic pressure in oscillatory blood pressure records. BERNARD FANTUS.

The shape of the oscillations may be of as great importance in determining the point of diastolic pressure in oscillatory blood pressure records

taken by the Erlanger method as the height of the oscillation. A compound lever tambour, such as the vertical membrane tambour of Zimmerman (Leipzig) or a 2.5 cm. tambour of Edward Meister (Johns Hopkins University, Baltimore), is more suitable for expressing the shape of the oscillations than the simple tambour commonly used. To bring out the true shape of the oscillations, it is also necessary that an adjustable leak be provided in the tambour space, as suggested by Dr. O. M. Cope of the University of Michigan, instead of the minute hole in the tambour provided by Erlanger. A rather soft thoroughly elastic rubber bulb and an arm bag with a stiff leather backing are also indispensable requisites.

When these conditions are met, it will be found that *the pulse oscillation that would make a triangle of greatest area* coincides with the fourth phase of Korotkoff. Under optimum conditions, a striking change appears in the succeeding oscillation, consisting of a lack of support in the downstroke of the pulse oscillation, causing a more definite expression of the dicrotic notch. This change is evidently due to the fact that the tambour lever flung up by the pulse wave is now no longer well supported during its descent, owing to the flaccid condition of the elastic system. Hence, the last well supported downstroke in the oscillation that would make a triangle of greatest area indicates the level of diastolic pressure. In experimental procedure a signal magnet marks the Korotkoff sounds, which were registered by an observer, without looking at the tracing that was being taken. The first sound of Korotkoff is indicated by one tick of the signal magnet, the fourth phase is signalled by two ticks, which are lettered "D." A monometer registers the pressure in the arm bag synchronously with the oscillatory record of an Erlanger bulb and tambour arrangement the tracing of which indicates the systolic criterion of Erlanger and the diastolic criterion here described. The carotid tambour record taken synchronously (breath being held) shows by the time relations that the marked notch following "D" in the Erlanger bulb and tambour tracing is the dicrotic notch.

The preanacrotic phenomenon and its relation to the arterial compression sounds of Korotkoff. A demonstration. JOSEPH ERLANGER.

Records obtained from the artery in situ where and while it is being decompressed[1] as in estimating the arterial pressure in man, show a series of small waves, the most prominent of which usually is negative, immediately ahead of the anacrotic limb of the pulse, but fully developed only in the more distal parts of the compressed length of artery. Korotkoff sounds first become audible in the artery beyond the compression simultaneously with the development of these waves. As decompression proceeds, these waves increase in amplitude and complexity, but disappear, usually quite abruptly, with the first pulse of the fourth sound-phase and when the artery first remains fully rounded but undistended at the end of diastole. During this first-to-fourth sound-phase

[1] Erlanger: This Journal, 1917, xlii, 588.

period the anacrotic limb of the pulse steepens as it proceeds along the compressed segment. These changes in pulse form we designate the preanacrotic phenomenon.

A similar phenomenon is seen in a model consisting mainly of a water-filled tube about one meter long, rolled out of thin rubber dam, through which artificial pulses are propagated. When this tube is partially filled, for instance so as to be half flat, the anacrotic limb of the pulse, as it proceeds, for a time steadily increases in steepness. About 7 to 12 cm. down the tube a preanacrotic negative wave begins to form. This wave deepens and shortens, and at about 15 cm. there appears in front of it a positive wave which also grows and eventually develops in front of itself another negative wave. At about 19 cm. the first negative and positive waves are found well up in the anacrotic limb of the pulse. This process then repeats itself over and over again some distance down the tube. The ascension of these *ripples* into the anacrotic limb is due to the faster propagation of the pulse proper. The same sequence can be traced in the records obtained from animals.

The position of ripple formation shifts downward as the tube fills, and at the degree of roundness (fullness) that is determined by a pressure of only 1-3 mm. Hg., ripple formation ceases. A lever resting across the tube is thrown violently from it, but only when the preanacrotic phenomenon is in evidence under it. A sharp snapping sound is produced by each pulse, but again only when and where the preanacrotic phenomenon is in evidence. Elsewhere and at other times if a sound is audible at all, it is dull in quality. Presumably, therefore, the sharp Korotkoff sounds are produced by the thrust of the steep anacrotic limb that develops in association with the preanacrotic phenomenon[1]. Sharp sounds are therefore heard in man during the stage of decompression included between the pulse following the first to penetrate the length of the compressed segment and the pulse following which the wall of the artery fails to be relaxed by the compression, that is, from just below systolic to just below diastolic arterial pressures.

Some uses of wire in the laboratory. ARTHUR D. HIRSCHFELDER.

Easily adjustable semi-rigid holders for cannulas, glass tubes, funnels, etc., can be made by twisting soft iron stove-pipe wire a few turns around a ring stand then twisting tightly into a double strand from 6 to 10 inches and then twisting a few turns about the object to be grasped. The double strand of iron wire is just rigid enough to hold in place many of the ordinary objects used in experimentation and yet flexible enough to admit of adjustment in every direction.

Spring brass wire of various weights can be used to make convenient retractors for operations and dissections. The wire is first bent into a "U" of desired size ranging from 5 to 15 cm. and the hooks to grasp the tissue are then made at the end of each arm of the "U." These also are

[1] Erlanger: Proc. Wash. Univ. Med Soc.; Journ. Mo. State Med. Soc., June, 1917, 258.

"U" shaped with arms 0.5 to 2 cm. in length bent at right angles to the arms of the large "U," with the open portion of the "U" pointing outward, the connecting arm of the "U" facing inward. In this way the small "U's" grasp the tissue on each side and are pulled apart by the spring of the large "U." The small "U's" may be made most quickly by heating the ends of the large "U" to take out the temper before binding. A spherical drop of solder placed at the free end of each small "U" prevents the retractor from tearing the tissues. Retractors made in this way are very satisfactory.

A convenient lever for marking signals, recording contractions of muscles, ventricles, etc., can be made from spring brass wire by winding one end into a coil around the ring stand and leaving the other end to extend horizontally out from the ring stand for 15 or 20 cm. This horizontal arm forms the recording lever. A small ring twisted into the other free end of wire coil can be twisted into place directly below the horizontal lever arm and a thread can be run through this ring and attached to the lever arm. This thread may be pulled on directly or it may be brought out through a second loop of wire attached to the base of the ring stand. A pull upon the thread causes movement of the lever, which can be recorded on the drum.

Some simple valves for respiration apparatus. ARTHUR D. HIRSCHFELDER and EDGAR D. BROWN.

A valve for use in respiration apparatus is easily made by taking a ½ or 1 ounce tin salve box, and cutting a hole in the top and one in the bottom of the box, and into the hole soldering a piece of brass tube. Care must be taken that the end of the tube does not protrude into the salve box. An elliptical piece of thick rubber dam, a little larger than the hole, is now placed so as to cover the hole and is held in place by a U-shaped bridle of spring brass wire whose ends are soldered down upon the inside of the salve box. For respiration experiments two such salve boxes are used—soldered upon each arm of a brass T-tube, the lid of one box on arm of the T, the body of the other box upon the other arm. In this way an inflow and an outflow valve are made. The apparatus is very easily constructed and the valves work well.

A simple stalagmometer. ARTHUR D. HIRSCHFELDER.

A piece of fine brass wire is inserted into the end of a piece of thick walled barometer tubing of 2 mm. lumen and 7 mm. outside diameter. The end of the tubing is closed by fusing in a small blast flame and the wire is then fused into the glass for a distance of about 15 mm. by gradually advancing the blast flame along the tube. It is then allowed to cool and a bulb to hold 2 to 3 cc. is blown into the tube 20 or 25 cm. away from the fused end. The fused end is now ground off square with a grindstone taking care to keep the grindstone moist. The coil of wire is now dissolved out. For this purpose a very fine capillary pipette is drawn out filled with nitric acid and introduced through the barometer tubing clear down into the fused portion, so that the acid comes into

contact with the upper end of the wire. The whole barometer tube is now placed in a test tube containing concentrated nitric acid 5 or 6 cm. deep so that the wire is acted on by nitric acid from within and without the barometer tubing. The test tube is now placed in a water bath and left there one or two days, after which the wire coil will be found to have been completely dissolved out and a fine capillary bore left in its place. If wire of no. 28 B. and S. gauge is used a stalagmometer which is very good for ordinary purposes may be prepared discharging 9 to 60 drops per minute. If wire such as is used for obturators of fine hypodermic syringe needles is used a very fine capillary is obtained delivering one drop in from twenty to thirty-six seconds. Any degree of frequency can thus be obtained.

An experiment for training students in the technique of intravenous and intraspinal injection. ARTHUR D. HIRSCHFELDER.

A human upper arm and forearm is obtained from the dissecting room, and a flap of skin dissected back for a distance of 10 cm. above and 10 cm. below the elbow on the pronator surface of the arm. An artificial vein is formed by taking a segment of rabbit's small intestine and connected by means of glass and rubber tube with a pressure bottle or funnel filled with water or colored fluid at an elevation of about 20 cm. above the table. The distal end of the intestine is closed off with a clamp. The filled segment of intestine is now introduced under the skin of the arm along the course of the median basilic vein and the skin flap is replaced and held in place with clamps. The distended segment of intestine presents under the skin in much the same appearance and position as the vein in man; and the student can now practice inserting a syringe needle into it, drawing up the "blood" and injecting any desired liquid into the "vein." The deeply seated veins of a fat individual can be simulated by interposing a thin layer of fat which can be kept in alcohol in a specimen jar. A sclerotic vein can be simulated by using a piece of white rubber tubing instead of the rabbit's intestine.

Practice in intraspinal injection may be afforded by two procedures. For preliminary practice, the spinal column of a human skeleton is articulated upon a bent brass rod. The segments may be held in place with modeler's clay or plasticin. Modeler's clay is now placed around one side of the vertebrae to simulate the tissues of the back. The student can now learn the sensation of introducing the needle between the bony structures of the vertebrae by introducing a stiff wire into the spinal canal. Having acquired the desired proficiency he can then practice upon the second model. This consists of the lumbar and sacral portions of a human trunk, screwed upright upon a piece of inch board which is just large enough so that, when in use it can be clamped to the table, and when not in use the whole specimen can be placed in a specimen jar filled with alcohol. Upon the left side of the specimen the muscles and fascia are dissected off along the spinal column so as to leave only the interspinous ligaments in place. The skin is left intact. A wedge of bone is sawed out along the anterior aspect of the

centra of the vertebrae, so as to expose the spinal canal. The student can now practice inserting the needle. He can observe upon the dissected left side exactly the bony and ligamentous structures through which his needle is passing and at the front of the vertebrae he can see exactly how and where his needle has entered the spinal canal and its exact relation to the spinal cord and the cauda equina.

The proportionate measurements of two hundred and fifty full term newborn infants. R. TAYLOR.

The results of the comparative measurements of 250 normal, full term, newborn infants show that from finger-tip to finger-tip is further than from crown to heel; that the occipital frontal circumference is barely greater than the sitting height, but decidedly out-measures the chest circumference; that the trunk length is greater than the arm, and the latter longer than the leg.

As regards individual variations, the spread of the arms is as long or longer in 81 boys and 82 girls, 65 per cent of the total. The head circumference is greater than the chest in 119 boys and 120 girls; the trunk length greater than the arm length in 119 boys and 123 girls, and greater than the leg length in 123 boys and 124 girls. The arm length exceeded the leg length in 111 boys and 107 girls. In 114 boys the mid point of the body lay at or above the navel and below in 11, the extreme figures being 32 mm. above and 10 mm. below. It was at or above the navel in 100 girls and below in 25. They showed greater variations, the extremes being 36 mm. above and 14 mm. below. The proportionate lengths of trunk, arms and legs, the proportionate chest and leg circumferences and the position of the center of the body relative to the navel are diametrically opposed in the newborn and the adult.

The rôle of the afferent impulses in the control of respiratory movements. HELEN C. COOMBS and F. H. PIKE.

To an earlier statement[1] and our statement of last year on section of the dorsal roots of the spinal nerves[2] we now wish to add certain other facts.

Cats were used in our experiments. Ether and tracheotomy were routine procedures. A control tracing of both costal and abdominal respiration was taken by means of Crile stethographs attached to Verdin tambours. The subsequent procedure was varied. Our findings are:

1. Section of the vagi alone produces a slow, deep type of respiration which has often been noted.

2. Section of the dorsal roots of the thoracic and cervical nerves results in a diminution or cessation of costal respiration. The effect of section of both thoracic and cervical nerves is a more marked diminution of costal respiration than after section of the thoracic roots alone. Abdominal respiration remains unchanged after section of the thoracic roots and there is no marked alteration in the respiratory rate.

[1] Stewart and Pike: This Journal, 1907, xix, 328; Stewart: Ibid., 1907, xx, 407.
[2] Pike and Coombs: This Journal, 1917, xlii, 395.

3. Section of the brain stem below the anterior corpora quadrigemina results in an abnormal form of respiration. It becomes labored, with respiratory gasps initiated by the diaphragm. This type of respiration resembles that which prevails after anaemia of the brain during the period of resuscitation before the afferent impulses become effective.[1]

4. Section of the vagi, followed by section of the dorsal roots produces, (1) a slowing of the respiratory rate, and (2) a diminution of costal respiration with dyspnea ensuing in from three to five minutes. This may be more or less severe, depending on the location and magnitude of the sections of the dorsal roots. Such respiration lasts for from fifteen minutes to an hour, gradually fading out with no terminal gasps. In its later stages it resembles respiration after section of the posterior corpora quadrigemina and vagi.

5. Section of the dorsal roots followed by section of the vagi produces (1) diminution of costal respiration, (2) a slowing of the respiratory rate with a return of costal respiration. This nearly normal type becomes dyspneic in the course of time, and gradually fades out, as when the procedure is reversed.

6. Section of the posterior corpora quadrigemina, followed by section of the vagi, results in dyspneic respiration which fades out much more rapidly than when the vagi are intact.

7. Section of the dorsal spinal roots after section of the corpora quadrigemina produces no more severe effect than section of the corpora quadrigemina alone. That the effects of transection below the posterior corpora quadrigemina are not due to trauma or shock from stimulation of efferent inhibitory fibers follows from the fact that essentially the same picture results from nerve section alone.

8. Section of the dorsal spinal roots followed by section of the phrenics results in (1) diminution of costal respiration and (2) return of costal respiration when the diaphragmatic is put out of commission. If the vagi are then sectioned there is a total failure of respiration coming on in a shorter time than when the phrenics are intact.

Parallel determination of amylase and dextrose-glycogen of the blood, liver and kidney after feeding. E. E. Brown and C. W. Greene.

Sets of animals have been prepared and fed meals consisting primarily of carbohydrates, carbohydrates and fat, carbohydrates and protein, and protein (lean meat).

The blood, liver, kidney and muscle of these animals have been examined for carbohydrates by the Lewis-Benedict method and for amylase by the Meyers-Killian method. The animals were killed at intervals during the digestion and absorption of the test meal. The blood and tissue curves in the sets of animals showed the following: The curves of variation in sugar content after any meal containing carbohydrate increases to the eighth to twelfth hour of absorption then gradually declines to the normal. This curve is closely followed by the curves of

[1] Stewart and Pike: This Journal, 1907, xix, 328.

variation in enzyme content though the enzyme does not vary to so great a percentage amount. A meal of pure protein gives only a slight rise in carbohydrate content of the tissues examined. The amylase content of the liver after protein is not increased more than usual but the blood and the muscle both show a sharp increase. The liver shows the greatest increase in sugar during the cycle of carbohydrate absorption, it also exhibited the greatest augmentation of enzyme. The blood and the kidney present curves of slighter variation but of very constant character. No attempt has been made to determine the source of the enzyme observed.

Brain changes associated with pernicious anemia. HENRY W. WALTMANN.

While it has long been known that certain symptoms, such as numbness and prickling of the fingers and toes, ataxia, bladder disturbances, etc., referable to involvement of the central nervous system, may arise in the course of a pernicious anemia, it does not appear to be generally recognized, even yet, how constant these findings really are. Though Nonne, Minnich, and others who described the spinal cord changes present in this disease, could see no reason why these same alterations did not take place in the brain, it was not until 1913 and 1916 that Barrett described alterations in the brain analogous to the Lichtheim foci present in the cord. In addition to these foci, which are very characteristic, there is present in the brain also a diffuse parenchymatous degeneration of the medullary substance.

Many of these degenerative changes are intimately associated with the blood vessels, in the so-called peri-vascular space of which one often sees a hyalin-like material, probably containing a toxin, which extends into the areas occupied by the disintegrating white fibers. This would indicate that lymph stasis is an important factor in the mechanism of this destruction. The gray matter, particularly that of the convolutions, shows some pathological changes which also tend to support this view. Surrounding the pyramidal cells, one often notes a circular area of degeneration, the pyramids themselves showing all states of disintegration. It is possible that toxins present in Obersteiner's so-called pericellar space are responsible for this picture.

In conclusion, we might state that Lichtheim placques, analogous to those occurring in the spinal cord, are present in the cortex also, as Barrett reported.

Though only seven brains and cords were studied in this series, it would appear that these changes are present in the cortex in just as great a proportion of cases as appear in examinations of the cord, and that those who show a well marked subacute combined sclerosis of the cord are also the ones in whom these changes can be demonstrated in the cortex.

Relative to the mental disturbances associated with pernicious anemia it is generally recognized that well defined types of psychoses, such as manic-depressive insanity may occur in pernicious anemia patients which bear no relation whatever to this disease. The lesser disturb-

ances, such as irritability, somnolence, apathy, etc., may, however, be based, at least in part, upon the pathological lesions found in the cortex.

A study of the comparative anatomy of the biliary tract and the sphincter of Oddi with special reference to animals without a gallbladder. F. C. MANN.

The results of a previous research[1] performed at the suggestion of E. S. Judd, proved that definite changes were produced by removal of the gallbladder in dogs, cats and goats. These changes consisted in dilatation of all the extra-hepatic ducts, including the cystic duct when it was present. The results of other experiments seemed to show that this dilatation was due to the activity of the sphincter of Oddi.

The purpose of the present research was to determine, if possible, how animals without a gallbladder compensated for the lack of it. Several species, some with and others without, a gallbladder, were included in this study.

The following factors were investigated:

1. The diameter of the common duct.
2. The length of the common duct.
3. The point of entrance of the common duct into the duodenum.
4. Secretory pressure of the liver.
5. Tone of the sphincter of Oddi.
6. Histology of the sphincter of Oddi.
7. The walls of the common duct.

Of these the only points of difference noted so far were in the tone of the sphincter of Oddi and the thickness of the walls of the duct. In the animals without a gallbladder which have been investigated so far there does not seem to be very much tone in the sphincter of Oddi, and the walls of the duct seem thicker and stronger in these animals. The work is far from being completed.

Some further notes on the detoxification of potassium chloride in the guinea pig. S. AMBERG and H. F. HELMHOLZ.

After expressing the urine contained in the bladder guinea pigs of about 200 gram weight received intravenous injections of 2 and 3 cc. of a solution containing 5 per cent NaCl and 1.5 per cent KCl. Ten minutes after the injection only about 0.1 to 0.2 cc. of urine could be expressed from the bladder. The detoxifying action of the NaCl cannot be due to a hastening of the excretion of KCl. Previously reported experiments showed that injections of 5 cc. 5 per cent NaCl protected guinea pigs against 2 or 3 cc. 1.5 per cent KCl injected immediately afterwards, while 0.55 per cent NaCl did not protect. The possibility had to be considered that the injection of 5 per cent NaCl diluted the blood much more than 0.55 per cent NaCl. Haemoglobin determinations (Fleischl-Miescher) of the blood of guinea pigs were made before and after the injection of 5 cc. 5 per cent and 5 cc. 0.55 per cent NaCl. No marked differences were found in the effect of the 5 per cent

[1] Judd and Mann: Surg., Gynec. and Obst., 1917, xxiv, 437.

and the 0.55 per cent NaCl solution. The protective action of the 5 per cent NaCl solution therefore cannot be ascribed to a greater dilution of the blood. Guinea pigs of about 200 grams survive readily the intravenous injection of 2 cc. of a solution containing 50 per cent glucose and 1.5 per cent KCl. An intravenous injection of 2 cc. 50 per cent glucose leads to a more marked dilution of the blood than that of 5 cc. of the NaCl solutions. After such an injection the ear vessels are markedly dilated.

Development of certain types of malignant tumors of the thyroid. LOUIS B. WILSON.

The writer has in progress a pathological review of malignant tumors of the thyroid in man. These, while not of frequent occurrence are found to be much more frequent than is ordinarily supposed. They are often overlooked, especially in their early stages by clinicians, surgeons and pathologists, since many of the malignant types closely resemble both grossly and microscopically benign adenomas of "fetal" type. Both benign and malignant adenomas apparently have their origin in embryonic tissue which at first consists of relatively small cells with indistinct outlines and relatively large, round or slightly oval, densely staining nuclei. These cells are irregularly arranged and are separated only by scarcely discernible septa which consist essentially of thin-walled, flattened blood vessels. In the second stage the cells are arranged in cordon-like masses or long band-like platelets between which are the same "sinusoid" vessels. In the third stage the cordons or platelets are broken into roundish masses with the beginning formation of acini some of which may contain colloid. This stage is apparently the most critical. If the acini become well developed the tumor is apt to remain benign. If the acini are irregularly or imperfectly developed the tumor is apparently more apt to become malignant, if it is not already such.

A careful study of the histology of supposedly benign encapsulated thyroid tumors removed at operation in relation to these stages of development is important for the early diagnosis of malignant conditions.

Blood regeneration after simple anaemia. I. Curve of regeneration influenced by dietary factors. C. W. HOOPER and G. H. WHIPPLE.

In previous publications we have shown that the bile pigment secretion can be influenced at will by modification of the diet. We have shown that the curve of bile pigment secretion can be depressed below normal by a meat diet and can be raised much above normal by a diet rich in carbohydrates. A mixed diet in a healthy bile fistula dog is associated with a fairly constant mean bile pigment elimination. From the results of these experiments we have assumed that the liver may have a constructive ability in bile pigment formation which can be modified by diet, as well as the accepted eliminative function, which depends upon the destruction of red blood cells containing hemoglobin. Furthermore, we have considered the possibility that the liver may be concerned

in the formation of other body pigments than bilirubin; for example, hemoglobin.

At the present time we have very good evidence that blood regeneration, after simple anaemia of definite grade, can also be influenced at will by various dietary factors. The curve of blood regeneration on a meat diet is very rapid, a matter of days or a few weeks, while the curve of regeneration on a diet rich in carbohydrates is very slow, in some of the animals on a diet of bread and milk it has required months for complete blood regeneration.

Dogs of the bull mongrel type were used in all of our experiments. The following blood studies were carefully made a day before bleeding and at intervals varying from four days to a week after bleeding until complete blood regeneration had taken place. The hemoglobin was estimated according to the Sahli method. The blood was allowed to remain in contact with the $\frac{N}{10}$ hydrochloric acid exactly five minutes before diluting in order to insure accurate and comparable readings. The red blood cells and white blood cells were counted. The blood platelets were counted by the Ottenberg and Rosenthal method. Brilliant cresyl blue (dilution 1 to 300) was used for counting the reticulated red blood cells. Janus green (dilution 1 to 10,000) was employed to estimate the number of red blood cells containing mitochondria. Hematocrit readings of the percentage of blood corpuscles were made after centrifugation in graduated tubes for 3000 revolutions per minute for thirty minutes. The plasma volume was estimated by the introduction directly into the circulation of a non-toxic, slowly absorbable dye furnished by Dr. H. M. Evans. The dye was allowed to remain in the plasma long enough for thorough mixing and its concentration was determined colorimetrically by comparison with a suitable standard mixture of dye and plasma. The total blood volume was calculated from the plasma volume on the basis of the hematocrit readings. The principle of this method is similar to the Keith, Rowntree and Geraghty method for the determination of plasma and blood volume. The routine blood studies were always made before beginning an experiment. The animals were then placed on a bread and milk diet and on each of the following two days one-fourth of the calculated total amount of blood was aspirated from one of the jugular veins. The animals were then allowed to rest a day, and on the following day the routine blood studies were again made. Generally, the percentage of hemoglobin and the number of red blood cells were approximately one-half the normal. On this day the animals were placed upon the specified diets. Special care was taken in each case to give a diet containing a sufficient number of calories and amount of nitrogen to assure a positive nitrogen balance. The animals usually gained in weight.

The curve of blood regeneration on a diet consisting of lean scrap meat or beef heart was very rapid. Out of eleven dogs, ten showed complete blood regeneration in from two to four weeks. Usually the hemoglobin and red blood cells regenerated with equal rapidity. Several splenectomized animals made anaemic and placed on lean meat diets

regenerated as rapidly as normal animals. One bile fistula dog fed on a beef heart diet showed complete blood regeneration in three weeks.

In the next series of experiments we studied the curve of blood regeneration on diets rich in carbohydrates. The first group of dogs was fed a diet consisting of white bread and milk; the second group a diet of cracker meal, lard and butter; the third a diet of white bread, lard and butter. The curve of blood regeneration on each of these diets was very slow, requiring from four weeks to five months for complete blood regeneration.

In some of the animals, especially those on a bread and milk diet, the curve ascended slowly for a short initial period of a few weeks, when the animals lost weight, the hemoglobin fell and the anaemia progressed until they were placed on a meat or mixed diet. In some instances, the number of red blood cells increased very rapidly and within three or four weeks after the initial bleedings the total number of red cells was even greater than at the beginning of the experiment. However, the cells were small and fragmented. The percentage of red blood cells as indicated by the hematocrit readings was the same as on the days immediately following the initial bleedings. In other words, the increase in red cells was only relative, due to the development of small and fragmented forms.

Simple bile fistula dogs made anaemic and placed upon a bread and milk diet reacted the same as the normal dogs.

However, splenectomized dogs made anaemic and fed a bread and milk diet or a diet consisting of cracker meal, lard and butter, generally regenerated slowly for a period of from four to seven weeks when a sudden recidivation developed resulting in death within a very few days. In some cases the hemoglobin fell from 95 per cent to 24 per cent, the red blood cell count from six million to one million within a week without jaundice. Furthermore, there was always a leucocytosis and a diminution in the total blood volume. The anaemia was usually of secondary type in the animals that retained their appetites and usually of primary type in those that refused to eat. This latent anaemia period was always accompanied by an intense urobilinaemia without a noticeable rise in the bilirubin content of either the blood plasma or urine.

The curve of blood regeneration on a mixed diet consisting of meat, bread and milk, or meat, cracker meal, lard and butter was quite rapid, usually resulting in complete blood regeneration in from three to five weeks. Splenectomized dogs reacted on this diet as rapidly as the normal animals. One simple bile fistula dog made anaemic and fed a mixed diet recovered completely in three and a half weeks.

Iron in the form of Blaud's pills, administered daily during the anaemia period had no appreciable effect on the curve of blood regeneration in any of our experiments.

Blood regeneration after simple anaemia. II. Curve of regeneration influenced by starvation, sugar, amino acids and other factors. G. H. WHIPPLE and C. W. HOOPER.

These experiments were performed under conditions described in the preceding communication. The dogs were kept in metabolism cages, food and water being given by stomach tube every twenty-four hours after catheterization. The urine was analyzed for total nitrogen.

Starvation. A curve of blood regeneration may be formed by multiplying blood volume by the hemoglobin percentage. This curve of *pigment volume* will show a drop to about one-third normal after the unit bleeding. During the first week of repair there will be usually a small but definite rise in the curve—perhaps a rise of 10 per cent. The following weeks (3 to 4) will usually show a slow but steady rise of 5 to 20 per cent of the total pigment volume. This reaction may be observed in normal dogs, bile fistula dogs or splenectomized dogs, and we have no evidence of any marked differences in blood regeneration under these experimental conditions. It is interesting to note the same speed of blood pigment regeneration in a bile fistula dog with bile pigment excluded from the intestine, proving conclusively that the body does not rely on absorption of any pigment substance from the intestine (bile pigment, urobilin or urobilinogen).

Sugar feeding. The experiments are in every way similar to those just described but for a definite amount of cane sugar or glucose or a mixture of the two sugars (50 to 125 grams) dissolved in a uniform amount of water and given by stomach tube. The curve of pigment volume or blood pigment regeneration may be followed each week as before and will show some differences from the starvation curve. We note the same definite rise during the first few days or first week—a rise of 10 per cent of the original pigment volume, but this is usually followed by a fall of 2 to 5 per cent during the second week and minor fluctuations after this for the usual period of three to four weeks. In other words, after the initial rise in the first week, the dogs fed on sugar show no gain in blood pigment and often a slight loss in contrast to the starvation experiments which show a small but definite gain.

It is to be noted that the protein decomposition in the body as indicated by the urinary nitrogen elimination is much less in the dogs fed on sugar than in the starved dogs. Both groups of dogs are receiving no nitrogen by mouth and yet they are able to form hemoglobin and red corpuscles during this interval. How much new red blood cell construction is going on during these periods we cannot say, but surely there is some disintegration of red cells to be made up and the curve of pigment volume shows a definite rise, more pronounced in the starving dogs which of necessity are excreting more nitrogen and breaking down more body protein. We are forced to the conclusion that the body conserves certain substances from this body protein autolysis and uses them over again to construct red cells. We have evidence that a part of this conservation is due to liver activity. There is no evidence for increased protein katabolism.

The initial rise in the pigment curve during the first week is not easy to explain, but we have assumed that the body has stored in reserve certain necessary substances which are used in this emergency to form new red cells, but this reserve is rapidly depleted and we have experiments to show that the red cells formed in such emergencies may not measure up to the usual standard and may fail the body in service.

Gliadin feeding influences the curve of pigment regeneration no more than sugar feeding, and is associated with only minor fluctuations in the pigment volume. Four or five weeks of gliadin feeding may show the pigment volume at the same level as at the start.

Hemoglobin (dried washed red cells, 10 grams per day) added to a sugar diet will cause a definite rise in the curve of pigment regeneration, but the same amount of dried meat protein might give a similar reaction.

Gelatin feeding has a striking influence upon the curve of pigment regeneration and red cell formation. Gelatin added to a sugar diet will usually cause a sharp rise in the curve of pigment regeneration. This rise is not as marked as after meat feeding. It usually reaches its maximum after two to four weeks, and then may show a fall coincident with malnutrition which may develop after prolonged gelatin feeding. Gelatin combined with cracker meal and lard may show a complete regeneration to normal within three weeks. This may be followed by a fall below normal if the diet is continued several weeks.

Through the coöperation of Doctor Van Slyke, Doctor Rohde and Miss Foster we have been able to show that the monoamino acid fraction of gelatin hydrolysis can influence in a striking manner the curve of pigment regeneration—almost as efficiently as whole gelatin. However, the diamino acid fraction of gelatin has a similar effect, but little less marked. Histidine (1.5 gram per day) combined with a sugar diet has no notable influence on the curve of regeneration—perhaps a trivial increase. We can submit no evidence that any single amino acid or group of amino acids is the single essential factor in this reaction which determines the curve of pigment construction and regeneration of the red cells. It seems very probable that several diet factors are concerned—as well as body conservation of certain elements of protein katabolism. It is suggestive that a meat diet gives a maximum hemoglobin production and a minimum bile pigment secretion in the same bile fistula dog. Also, a bread and milk diet gives a minimum hemoglobin production and a maximum secretion of bile pigment in a bile fistula dog. It is possible that some substance in the meat enables the body to conserve or fix and reconstruct certain substances resulting from tissue katabolism. The amount of body protein autolysis or katabolism certainly is an important factor. There is a constant wastage of pigment substance (bile pigment) and a uniform construction of pigment substance in the body even during starvation. The products of protein katabolism in the body as well as dietary factors contribute to the steady construction of body pigment, especially hemoglobin.

Changes in reflex thresholds following shock from manipulation of the intestines. EUGENE L. PORTER.

Shock was produced in spinal cats by exposing and manipulating the intestines. In about 50 per cent of the animals the threshold of the flexion reflex was raised by this procedure in from one to five minutes. In the remaining animals no change could be detected. Commonly the new threshold was 30 per cent to 50 per cent higher than the original; the maximum observed was a 100 per cent increase. The high threshold was maintained as long as manipulation continued (ten to forty minutes). Upon returning the intestines to the abdominal cavity the threshold usually dropped, within about ten minutes, nearly or quite to its original level. In some cases it was possible to repeat the experiment on the same animal. There was no parallelism between the changes in reflex threshold and changes in blood pressure.

Note in regard to the amount of sugar normally in the blood of cats. ERNEST L. SCOTT.

In 1914 the author published a fairly extended account of the amount of sugar to be found in the blood of the domestic cat under ordinary laboratory conditions. At that time comparisons were based upon the average obtained from twenty-four cats which were prepared in a manner carefully described. Animals so prepared and killed by decapitation were found to vary but little from this average which was 0.069 gram of sugar per 100 grams of blood.

Since that time numerous determinations of glucose in the blood of cats which were prepared in a manner essentially similar to that adopted for the standard animals in the above series have been made.

In all, determinations upon something over one hundred such animals have now been made and while there is a slightly greater range of variation, the average for the longer series remains at the same place, 0.069.

On the comparative absorptive power for drugs of the bladder and urethra (male). DAVID I. MACHT.

In other communications dealing with the absorption of drugs from the conjunctiva[1] and from the vagina,[2] published elsewhere, the author called attention to the fact that apomorphin, by virtue of its being a centrally acting emetic, furnishes a convenient means of demonstrating absorption of drugs through unusual channels. If a 1 per cent solution of apomorphin hydrochloride is introduced into the bladder of a male dog through a hard catheter, the latter instrument being allowed to remain in place, the solution remains in the bladder and owing to the powerful spasmodic contraction of the urethral sphincter in the male dog, practically none of the drug gets into the urethra. Under these circumstances vomiting does not occur sooner than half an hour after the introduction of the poison and sometimes after the lapse of an hour

[1] Journ. Amer. Med. Assoc., 1917, lxviii, 1230.
[2] Journ. Pharm. Exper. Therap. (in press).

or more, and very often not at all unless the catheter be removed. If, on the other hand, the urethra of the same dog, on another day, be irrigated with the same solution or even weaker solutions of apomorphin, care being taken not to inject the drug into the bladder but to confine the irrigation only to the urethra and allow the fluid to run back, vomiting is produced in every case in from three to five minutes. Inasmuch as vomiting is produced in dogs almost as efficiently by means of morphin as with apomorphin, the same results can be obtained by using that alkaloid. Even strong solutions of morphin confined to the bladder produce either no vomiting at all or only after the lapse of a considerable period of time (half an hour to one hour). On the other hand, the introduction of a little morphin solution into the urethra is followed in the dog by vomiting in a few minutes. The remarkable difference in the absorptive power between the urethra and the bladder noted after morphin and apomorphin, holds good for a large number of other drugs and poisons. The author has studied in this connection the effect of various alkaloids, a number of antiseptics, some local anesthetics and a number of salts. The complete account of the investigation will be published in due time in the *Journal of Urology*. It may be stated in this place that an inquiry into the absorptive power of the ureters is also under investigation by the author.

A gradient of metabolism in the intestinal muscle. WALTER C. ALVAREZ.

There is a gradient in rhythmicity from the duodenum to the ileum, the rates, in the rabbit's intestine, varying from 15.3 per minute near the pylorus to 10.5 per minute near the cecum. There are also gradients of irritability and latent period. In the dog, the duodenal muscle responds to a strong stimulus after 0.13 seconds, the ileum after 0.22 seconds.

The best explanation for these differences is that there is an underlying gradient of metabolism. Child has shown that the regions with high rates of metabolism and faster oxidation suffer more from the action of weak solutions of KCN than do the regions with slower rates. Thus in the Ctenophore mnemiopsis there is a gradient of susceptibility to KCN in the conducting paths along the rows of swimming plates. The pace-maker region suffers so much more than the others do that the impulse may at times be even reversed.

I have caused five segments from different parts of the intestine to beat rhythmically in the same beaker of Locke's solution. The addition of 1 part of KCN to 1,300,000 parts of the solution caused a marked loss of tone and rhythmicity in the duodenum and jejunum, while the ileum and colon were much less affected. Some seventy-five drugs have been tried out, and several show similar differences in their action on different parts of the gut.

Simply shutting off the air bubbling through the solution has often been enough to show this graded effect down the intestine The duodenum generally suffered most; the jejunum came next, and the two segments of ileum and the colon suffered least.

These observations, so comparable with those of Child, furnish considerable proof of the presence of a metabolic gradient down the intestine. The practical aspect to the problem is the fact that the results obtained suggest strongly that some of the emetics and purgatives owe their effects to alterations in this gradient—alterations brought about by an unequal or dissimilar action of the drugs on the two ends of the tract.

Studies on cholesterol. V. The blood cholesterol in malignant disease and the effect of radium treatment on the blood cholesterol. GEORGINE LUDEN.

Between November, 1915 and December, 1917 a total of 1069 samples was tested for cholesterol. The above figure includes 1052 determinations of blood cholesterol on human blood, goat's blood, gopher's blood and dog's blood; 14 determinations on foodstuffs and 3 on human pus. Of 743 blood samples parallel determinations in triplicate were made with Bloor's original method (Bloor I, with sodium-ethylate) and its modification (Bloor II, without sodium-ethylate) making a total of 4658 determinations with Bloor's methods. The advantage of these parallel determinations by which the amount of cholesterol split-products present in the blood is revealed, was shown by tests made on pathologic human blood, including 70 miscellaneous conditions, 41 cases of pernicious anemia, 37 of exophthalmic goiter, 3 of myxedema tested 18 times at various intervals during the administration of the thyroid hormone (Kendall's thyroxin), 79 determinations on the writer's own blood as normal control, 9 cases of sarcoma, (16 determinations) and 92 determinations of the blood cholesterol in carcinoma before and after radium treatment including 20 weekly determinations on one patient.

The blood cholesterol values in carcinoma were found to be high in 43 per cent of all the patients and in 56 per cent of those that were to have radium treatment. In the latter 54 per cent had equal values with the Bloor I and Bloor II method. These equal values indicated some disturbance of cholesterol metabolism in carcinoma, since a difference between the values obtained by the two methods is always found normally and no equal values were found in 252 determinations on non-malignant cases. After radium treatment the equal values were found to disappear and the percentage of high values dropped to 10 per cent. In sarcoma the blood cholesterol values were found to be very much lower than in carcinoma, equal values were observed in two cases also and the effect of radium was similar to that observed in carcinoma.

In myxedema high cholesterol values (but not equal values) were found and the administration of the thyroid hormone brought the blood cholesterol back to normal at a rate parallel to the rise in basal metabolism which it induced.

These observations seem to warrant the following conclusions:

1. That the high blood cholesterol values found in carcinoma are

not due to cell destruction, since they are lowered by radium treatment although radium causes cell destruction, but that they are due to a disturbance of cholesterol metabolism.

2. That the disturbance of cholesterol metabolism may be but evidence of a low rate of basal metabolism, since the high cholesterol values in myxedema are reduced by the administration of the thyroid hormone by which the rate of basal metabolism is greatly increased.

3. That radium treatment, by lowering the blood cholesterol values, alters the chemical composition of the blood, a fact which has not hitherto been taken into account in the study of the effects of radium treatment.

4. That the administration of the thyroid hormone affects the blood cholesterol values in a manner similar to that of radium treatment and may therefore be expected to be equally beneficial to patients suffering from carcinoma. However, careful investigation (in progress by the writer) will be needed before definite conclusions can be drawn concerning the effect of thyroxin in carcinoma.

Accuracy or Guesswork—

WHICH?

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